EXAMINING HESSIAN FLY (*MAYETIOLA DESTRUCTOR)* MANAGEMENT CONCEPTS AND QUANTIFYING THE PHYSIOLOGICAL IMPACT OF HESSIAN FLY FEEDING ON POST-VERNALIZATION SELECTED CULTIVARS OF WINTER WHEAT IN KANSAS

by

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B.S., Texas Lutheran University, 2005
M.S., Kansas State University, 2008

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

The Hessian fly, *Mayetiola destructor* (Say), has been a historically significant pest of wheat in Kansas. However, it has been 60+ years since research has been conducted examining the flies' activity throughout the year. Results of pheromone trapping in 4 counties in Kansas shows that Hessian fly (HF) males are actively flying in the fall, at least 1 month after the historical fly-free dates. Therefore, the Hessian Fly-Free Date is no longer valid and should be referred to as the Best Pest Management Date. Using pheromones for fall and spring trapping also indicated that HF is more active throughout the spring than previously thought, with almost continuous fly emergence and numerous emergence peaks in both spring and fall. The use of resistant wheat cultivars has been adapted to protect seedling plants from HF larval feeding in the fall. However, it is unknown if these cultivars are still providing protection after winter vernalization. Greenhouse trials indicated that 'Armour', a cultivar considered intermediately resistant, remains resistant under infestation levels of 1 fly/tiller but significant seed weight losses occured under infestations of 3 flies/tiller. In the field, Armour did not provide protection post-vernalization, with plants containing similar numbers of flaxseeds (pupae) as the susceptible cultivar, 'Fuller', and having significant losses of culm height (cm), number of spikelets/spike, number of seeds/spike, and seed weight (grams) when infested. 'Duster', a cultivar considered highly resistant, appeared to provide resistance to HF larval feeding in both the greenhouse and the field, and even produced significantly heavier seeds when infested with 3 flies/tiller in the greenhouse. These results suggest that post-vernalization screening should be conducted on all HF resistant cultivars to determine if each continues to provide protection. Little information is available showing if and how HF larval feeding on more mature wheat (Feekes 7-10), post-vernalization, impact plants, aside from lodging. Greenhouse and field infestations of a susceptible cultivar, Fuller, showed that significant losses of culm height (cm), number of seeds/spikelet, and seed weight will result from as few as 1 larva /culm. Yield losses averaged 0.13g/spike (65 kg/ha) compared to non-infested plants.

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Dedication

I would like to dedicate to my family. Without the endless support from all of you this would not be possible!

Chapter 1 - Introduction

Wheat

It is believed that wheat, *Triticum aestivum* L., was originally domesticated and cultivated over 7,000 years ago in Southwest Asia (Chapman 1992). In 2009, the Food and Agriculture Organization (FAO) of the United Nations estimated that over 600 million tons of wheat were harvested worldwide, making it the third ranking cereal crop in the world after maize (788 million tons) and rice (657 million tons) (FAOSTAT 2009). In the United States, Kansas is often referred to as the 'Wheat State' or the 'Breadbasket of the World', as nearly one-fifth of all U.S. grown wheat comes from this one state (Kansas Wheat Commission 2013). In 2012, it was reported that wheat was planted on 9.5 million acres in Kansas and produced 382 million bushels (Kansas Agricultural Statistics 2012).

Of the six classes of wheat grown in the United States, hard red winter, hard red spring, hard white, soft white, soft red winter, and durum, Kansas commonly grows three; hard red winter, soft red winter, and hard white. Hard red winter wheat is the dominant class, comprising 95% of all wheat grown, and can be found in every county in the state (Kansas Wheat Commission 2013). The ancestor of all U.S. grown hard red winter wheat is a cultivar called 'Turkey', a hardy plant that was introduced in Kansas by Mennonite settlers from the Ukraine in 1873 because it could handle Kansas' harsh weather conditions (Paulsen and Shroyer 2008). Today, there are over 60 hard red winter wheat cultivars available to producers (De Wolf et al. 2013).

Currently, there are several wheat cultivars considered highly resistant to Hessian fly, *Mayetiola destructor* (Say), including 'Billings', 'Duster', 'Gallagher', 'Iba', 'PostRock', 'T153' and 'T154' (De Wolf et al. 2013). Additionally, there are several cultivars which provide intermediate levels of resistance to the Hessian fly (HF), such as 'Armour', 'Hatcher', 'TAM 111', and 'Winterhawk' (De Wolf et al. 2013). However, these intermediate cultivars have demonstrated inconsistent results in greenhouse trials. These cultivars are screened for resistance in a greenhouse setting by planting seeds of wheat lines with different resistance genes or gene combinations and infesting them at the 1.5 leaf stage with adult HF females. Eggs are deposited and allowed to develop to the flaxseed (pupal) stage. Plants considered susceptible appear dark green and stunted while plants considered resistant grow normally and are a light green color (Chen et al. 2009a).

In 2013, Duster, a cultivar considered highly resistant to HF, was the leading resistant cultivar planted in Kansas, estimated to comprise 4.0% of all wheat acreage (Lingenfelser 2013). Due to its increasing popularity, this cultivar was chosen for this study to represent cultivars that are considered highly resistant to HF. Duster was developed and released by Oklahoma State University in 2006. This cultivar is ideal for the Southern Great Plains where it emerges well in hot, dry soil conditions and closes canopy quickly with abundant tillering (Edwards et al. 2007). While Duster has only intermediate straw strength, it is resistant to leaf rust and several viruses, but is considered unique due to its high resistance to the Great Plains biotype of HF, allowing growers to plant earlier to maximize forage yield or reap the benefits of no-till management.

The top three wheat cultivars of wheat planted in Kansas in 2013, 'Everest', TAM 111, and Armour (14.3, 10.8, and 6.7 % of total wheat acreage, respectively) are all currently rated moderately to highly resistance to HF, but all three have proven to have inconsistent resistance in greenhouse trials (De Wolf et al. 2013, Lingenfelser 2013). For this study, Armour was chosen to represent the cultivars with moderate and inconsistent resistance ratings. Armour was developed by WestBred® and licensed to AGSECO Inc. in 2008 and is considered broadly adapted for planting in the Central Great Plains including all of Kansas (WestBred® 2013). Armour tends to head out early with a long grain-fill period. It has an average head size but often puts out a large number of tillers, leading to a high yield potential (AGSECO Inc. 2013). While this cultivar provides excellent straw strength, tolerance to low pH soils and good resistance to leaf rusts and several viruses, it is considered moderately susceptible to HF (WestBred® 2013). In greenhouse trials Armour has shown inconsistent resistance to HF attack at the seedling stage (De Wolfe et al. 2013).

In 2010, when this study was initiated, 'Fuller' became the most popular cultivar of wheat planted in Kansas, accounting for 11.8% of the state's wheat (USDA 2010). Fuller, a cultivar considered susceptible to HF was developed cooperatively by the Kansas Agricultural Experiment Station and the Agricultural Research Service, USDA and was introduced in 2006. As of the 2013 season, Fuller is still one of the top 5 wheat cultivars grown in Kansas (Lingenfelser 2013) Fuller is most commonly compared to Jagger and shares many of its desirable traits such as early maturity, winter hardiness, and a high yield potential (Fritz et al. 2007). It is even considered superior to Jagger in respect to tolerance to shattering, test weight, and thousand kernel weight (Fritz et al.

2007). Fuller is considered highly to moderately resistant to several common wheat diseases and viruses but is considered moderately susceptible to powdery mildew and low pH soils and very susceptible to HF (Fritz et al. 2007).

The Hessian fly

The Hessian fly, *Mayetiola destructor* (Say), is an important pest of bread wheat, *Triticum aestivum* L., and durum wheat, *Triticum turgidum* L. subsp. Durum (Desf.) Husn., in North Africa, southern Europe, northern Kazakhstan, and North America (Lhaloui et al. 1992, Ratcliffe and Hatchet 1997, Martin-Sanchez et al. 2003). Bread wheat is a term referring to both red and white spring and winter wheat classes and the harvested wheat seed is finely ground and used for making bread products (Duke 1983). Durum wheat, on the other hand is used to make pasta, bulgur, couscous and is typically planted at the end of the winter and harvested in the summer (Donmez et al. 2000). Although the Hessian fly (HF) shows a preference for wheat, it is known to have a number of alternative hosts in the U.S., including cultivated and wild grasses in 16 genera of the grass tribe Triticeae (Zeiss et al. 1993, Chen et al. 2009b). Yield loss due to HF has been estimated in various parts of the world to have been as high as 32% and 42% (Amri et al. 1992, Lhaloui et al. 1992). This pest is becoming an increasingly frequent pest of winter wheat, *Triticum aestivum* L., in Kansas. It is primarily a pest in the eastern half of the state, but severe outbreaks have been reported in western Kansas (Sloderbeck and Whitworth 2009, Whitworth et al. 2009). Serious interest in HF in Kansas was sparked in 1908 when forty-one counties reported injury of 5 to 50% of their wheat crop, leading to the state-wide loss of approximately nine and a half million bushels (Dean and McCulloch 1915). The sporadic outbreaks of HF continued to lead to serious losses. An outbreak in Kansas in 1943 was estimated to have caused the loss of 25 million bushels of wheat (Horton et al. 1945). The HF was a frequent pest throughout the 1920's to 1940's but the development of the Hessian Fly-Free Date, resistant wheat cultivars, and cultural control methods diminished the number and severity of these outbreaks. In recent times, these outbreaks are becoming more frequent and have increased the interest in developing new resistant cultivars, re-examination of the HF-free date, and the re-emphasis of cultural methods to control this pest.

The HF is believed to have originated in Southwest Asia, where wheat originated (Barnes 1956). From there, fly populations spread west to the Mediterranean, north and west to Europe, and east to Siberia and central Asia (Hunter 2001). The insect gets its common name, Hessian fly, in the United States from the common, but unproven belief that it was brought to North America in the straw bedding of horses that belonged to Hessian soldiers from Germany that were hired by Britain, to fight against the American colonists during the Revolutionary War (Hunter 2001, Pauly 2002). By the late 1770's, the HF was a recognized problem in the New York area, was first reported in Kansas in 1871, and today, it is found in all major wheat growing areas of North America (Pauly 2002, Whitworth et al. 2009).

The adult is a small (2-4 mm long), fragile, dark colored, gall-making fly, (Diptera: Cecidomyiidae) with relatively long legs. They are short-lived (approx. 3-5 days) and do not feed. Females oviposit from 100-400 eggs on the upper surfaces of wheat leaves over a one to two day period (McColloch 1923). Mating is based on sex-pheromones released by the female (Harris and Foster 1999). Newly emerged females walk several centimeters from their eclosion site, assume a specific posture, release pheromone and continue to remain close to this location until they begin the active oviposition phase (Harris et al. 2001). During oviposition, females are constantly in motion, typically making short flights to ovipositional sites within a field (Harris et al. 2003). However, females are capable of diffusing over a 1500m^2 area in two hours when they emerge in a field without a host plant to oviposit on (Harris et al. 1993). Females are typically monogamous, which is caused by physiological and behavioral changes that occur after mating one time (Foster et al. 1991a, Bergh et al. 1992). In contrast, males are constantly moving, finding and mating with numerous virgins until they die; one male may fertilize as many as 3,445 eggs in his short lifetime (Bergh et al. 1992).

The tiny, reddish, oblong eggs may look similar to the early stages of wheat rust and are typically deposited in the grooves on upper surfaces of leaves, but are so small they cannot be readily seen without a hand lens (Dean and McCulloch 1915). In three to 10 days, depending on the temperature and humidity, reddish maggots will emerge and the first instar larvae crawl to the base of the plant where they feed between leaf sheaths at the crown in seedling wheat plants or at one of the joints in adult wheat plants (Dean and McCulloch 1915, McCulloch 1923, Ratcliff and Hatchett 1997). Foster and Taylor (1974) demonstrated the optimal temperature for growth, developmental time, adult emergence and maximum offspring per female is 21.1°C. The larvae go through three instars. The first instar lasts approximately five days at 20°C and is the most important stage for survival of the fly as it must successfully establish a permanent feeding site so it may develop into the immobile second instar, which will become the non-feeding, pre-pupal third instar (Byers and Gallun 1971). The first and second larval instars feed and cause damage to wheat plants (McCulloch 1923, Ratcliffe and Hatchett 1997). Plants infested in the seedling stage may increase tillering in an attempt to compensate for damaged tillers (Anderson et al. 2011). However, feeding has been shown to reduce the elongation of the 2nd leaf by over 59%, almost completely stunt the third leaf, and prevent further leaf development and or initiation of leaves as the shoot apical meristem, the site of cellular growth and differentiation, dies (Cartwright et al. 1959, Byers and Gallun 1971). Wheat attacked during stem elongation may have similar symptoms and is also susceptible to lodging, and if it doesn't lodge, may cause production of heads with lower seed weight and fewer seeds (Ratcliffe and Hatchett 1997).

In Kansas, at the time this study was initiated, it was thought that there were typically two main HF broods/generations per year, one in the spring and one in the fall although there may be late spring, mid-summer, and late fall supplementary broods of varying sizes (Byers and Gallun 1971, Whitworth et al. 2009). According to Dean and McCulloch (1915), in Kansas, adult flies begin to emerge from the overwintering flaxseeds about the first of April and may continue to emerge until the last of April. They also report the main fall brood emerges sometime during the last of August until the middle of October, with emergence most often occurring in September. Although they did note that, under favorable conditions, a brood of flies could appear midsummer and develop in the volunteer wheat, or in late November, little significance was given to these potential populations (Dean and McCullough 1915). In general, HF actively develops in the spring and fall when temperatures are mild. Summer and winter are spent inside plant sheaths in a puparium commonly called a 'flaxseed'. When summer temperatures reach above approx. 30 - 36°C, HF will aestivate until temperatures remain at or below 21.1°C for four or more consecutive days (Wellso 1991). In the laboratory, when temperatures are decreased below 4°C and remain cold for several weeks, pupae will enter diapause and will remain in diapause for at least 70 days (Dendy 2009).

Feeding by the fall brood of larvae may stunt wheat seedlings, may kill them outright, or may make them more susceptible to winter kill. Spring brood larvae prevent the heading of small tillers, cause stems to lodge, and reduce the weight of the grain (Hill and Smith 1925). The physical interaction between the wheat plant and HF is initiated when first instar larvae insert their mandibles into plants. Mandibles are minute, blade-like structures that taper into sharp, pointed teeth, apparently adapted for piercing plant cell walls (Hatchett et al. 1990). These mandible blades appear to be capable of piercing the epidermal cell wall but do not reach the plasma membrane of the plant cell (Stuart et al. 2012). HF mandibles are grooved with a canal-like opening which connects to the salivary glands, suggesting this opening is used to transport salivary secretions, into, or just below the cell wall of a host without actually disrupting the plasma membrane (Harris et al. 2006, Shukle et al. 2008). Salivary glands are composed of two regions: the large basal region, which is directly connected to the mandibles and is the primary source of salivary secretion and injection in the first instar larvae, and the distal filamentous region (Hatchett et al. 1990). This basal region decays at the beginning of the second instar once the larvae have established a feeding site and have irreversibly transformed the plant into a permeable nutrient sink (Stuart and Hatchett 1987, Williams et al. 2011). Intense study of the salivary glands of first instar HF larvae found signal peptide-encoding transcripts that encode putative effector proteins called secreted salivary gland proteins, or SSGPs (Chen et al. 2004). While the role that these SSGPs play in the HF and wheat interaction remains to be determined, there is little doubt that at least some of these genes encode effector proteins that modulate plant development (Stuart et al. 2012).

Early studies showed a five to six day lapse between the cessation of HF feeding and resulting damage of the plant, suggesting that larvae secrete something into the plant that interferes with production and movement of plant metabolites rather than damaging the plant by removal of plant juices (Byers and Gallun 1971). More recent studies have demonstrated that feeding by HF larvae leads to dramatic changes in the metabolic pathways of wheat plants, reducing the plant's ability to grow and develop normally (Anderson and Harris 2006, 2008; Harris et al. 2006, 2010; Zhu et al. 2008). Epidermal and mesophyll sheath cells close to the larval feeding site become what are called nutritive feeding cells and have an enriched cytoplasm, altered nucleus, and a thin cell wall which can break down to produce a liquid diet for the developing larva (Harris et al. 2006). In addition, plant cell division and cell elongation stop and chloroplasts, carotene, and xantophyll accumulate, giving infested plants a dark green color (Miller et al. 1958, Cartwright et al. 1959, Robinson et al. 1960). These physical symptoms are related to changes in plant gene transcription including the upregulation of genes encoding stress proteins, transcription factors that modulate plant development, and genes involved with primary metabolic pathways (Giovanini et al. 2006, Subramanyam et al. 2006, Liu et al. 2007, Wu et al. 2008, Zhu et al. 2008,). At the same time, many plant genes are down regulated, including many plant defense genes and genes encoding structural proteins needed for plant growth (Liu et al. 2007, Saltzmann et al. 2009). These new metabolic pathways produce more amino acids needed for HF nutrition and produce nutritive cells at the larval feeding site (Liu et al. 2007, Zhu et al. 2008).

The life history and biology of the HF makes it a difficult pest to control. Once an infestation is established there are currently no remedial measures that can protect the crop (Whitworth et al. 2009). Instead, HF management focuses on practices that decrease the ability of the fly to survive and reproduce.

Control Options for the Hessian Fly

Cultural Control

There are several cultural control methods that can be used to help reduce and prevent Hessian fly infestations. Growers are strongly encouraged to destroy infested stubble and volunteer wheat. Volunteer wheat, or wheat that emerges from seed left in the field from the last growing season, may allow HF to produce an extra brood which will then infest the planted wheat in higher numbers and may also reduce the effectiveness of other management practices (Whitworth et al. 2009). HF has been collected from volunteer wheat plants in large numbers, especially from plants that were allowed to remain present throughout the summer (Buntin et al. 1991). For the destruction of volunteer wheat to be most effective, it should be destroyed at least two weeks before planted wheat germinates (Whitworth et al. 2009). Even after infested wheat is harvested, pupating HF or flaxseeds, will remain in the stubble, and if undisturbed, may successfully over summer and infest wheat fields the following fall.

Tillage and Burning

HF abundance can be affected by tillage and burning practices (Chapin et al. 1992, Zeiss et al. 1993, Clement et al. 2003, Castle Del Conte et al. 2005). Plowing infested wheat stubble to bury larvae and therefore prevent adult emergence is a long established practice (Headlee and Parker 1913, McCulloch 1923). Zeiss et al. (1993) found that a post-harvest disking which buried stubble up to 9cm deep, but also left a small amount of stubble on the soil surface, resulted in at least 70% lower HF emergence the following fall compared to un-disked plots 75% of the time. However, reduction was only significant in one of the four experiments. An additional study conducted at the same location determined that burning alone did not reduce fly emergence (Zeiss et al. 1993). In the South Carolina Coastal Plain, it was found that while spring burning gave no reduction in fly emergence, a combination of spring burning and disking, to a depth of 10.9cm, reduced HF emergence 70-96% (Chapin et al. 1992). Plots disked to a depth of 9.6cm in the spring resulted in a 36-54% reduction in fly emergence. Plots disked only in the fall, to a depth of 11.4cm, reduced emergence by 48 – 50%. However, the number of flies that emerged between disked and non-disked plots was only significant in one spring and one fall of the two year study. Additionally, plots disked in the fall and spring showed no further decrease in HF emergence (Chapin et al. 1992). In one year of the study, a bottom-plowed treatment was added, plowing to a depth of 32cm. This deep tillage resulted in total elimination of fly emergence, suggesting that any tillage operation done to control HF emergence needs to be to a deeper depth than is typically gained by disking in order to reliably bury all flaxseed and thus eliminate fly emergence.

However, no-till and conservation tillage agriculture are practices that have been increasing in recent years. In Kansas, the number of wheat acres managed under a conventional tillage program decreased by anywhere from 13,000 to 57,000 acres in 18 of 22 counties surveyed from 2004 until 2010 (KSU Dept. of Ag. 2011). In no-till agriculture, the soil surface is not disturbed and crop residue is left on the surface at the time of seeding whereas, in conservation tillage, soil is typically tilled before planting, leaving some crop residue on the soil's surface (Stinner and House 1990). Leaving crop residue on the soil surface is the most economical way of conserving soil and moisture. However, the presence of surface residue may contain Hessian-fly infested wheat stubble and, even if populations were not large enough to be a problem in the previous season, may lead to higher population densities in the following wheat crop (Castle Del Conte et al. 2005, Wilhelm et al. 2007).

Insecticides

Insecticides have not been widely used to control HF because of the inconsistent efficacy they provide due to flies' short adult life span, the brief period the larvae are exposed on the leaf surface, and difficulty in reaching larvae in the leaf sheath of the plant. Proper timing of insecticide applications is very difficult, and the efficacy and cost effectiveness of these treatments is often not as high as other options. Buntin et al. (1992) demonstrated that while infurrow planting time applications of disulfoton and phorate provided positive economic returns under significant fall HF infestations, the use of high-yielding resistant cultivars alone provided similar or better economic returns. Repeated foliar applications of disulfoton (four to six sprays) were shown to reduce spring infestations up to 94% over untreated wheat and grain yield of these repeatedly sprayed plants was, on average, 463 kg/ha (approx. 6.9 bu/a) more than untreated plants in two of four years of this study (Buntin and Hudson 1991). Using these data, they were able to calculate, based on chemical and commodity prices at the time, that three applications of disulfoton could be economically justified. During their study they were also able to show that for foliar insecticides to be effective, spraying must begin during stem elongation and before peak egg deposition. However, Buntin and Hudson (1991) concluded that overall inconsistent efficacy, marginal economic benefit, and difficulty of predicting HF ovipositional activity makes management of spring infestations of HF using foliarly applied insecticides unfeasible. Wilde et al. (2001) found that imidacloprid, thiamthoxam, and fipronil applied as seed treatments were all effective at controlling fall infestations of HF, but none were effective on a spring population of flies, thus they did not show any insecticidal activity after vernalization. This study also concluded that the cost of applying an insecticide to wheat seeds is economically prohibitive unless HF densities are locally significant and a chronic problem in a given area.

Fly-Free Date

One of the major methods used to avoid fall HF infestations is to plant wheat late enough in the season that adult HF have emerged and completed oviposition before wheat has emerged. Many states in the eastern Midwest have established fly-free dates, including Kansas. In 1915, the first fly-free date was published for Kansas. It was based on experimental data gathered from 1907 to 1914 (Dean and McCulloch 1915). Then, in 1918, a massive study was undertaken in Kansas that continued for 16 years. Throughout the state, wheat was planted in five-day intervals beginning around the second or third week in September and continuing for about a month. The wheat was monitored for HF infestation and yield data were collected (Horton et al. 1945). The information collected over these years was used to establish a more specific fly-free date for Kansas growers, a date after which HF were not active, but not so late in the season that yield was negatively impacted (Horton et al. 1945). These data vary across Kansas and the resulting fly-free date map has been widely published and is still used over 60 years later. Recent data collected with pheromone traps (Trécé Pherocon VI Trap, Adair, OK with PheroNet Hessian fly pheromone, Sweden) in Kansas indicates that HF adults may be active much later in the fall and spring than previously recognized and thus the previously accepted fly-free dates need to be revised (Davis et al. 2009). In addition, spring infestations may seriously impact wheat plant health and yields, even if fall infestations were absent or minimal.

Natural Enemies

While there are numerous parasitoids that attack Hessian flies in the United States, and many have been shown to cause significant mortality under laboratory conditions, there are no documented cases where they provide a consistent source of effective control in the field (Gahan 1933, Hill et al. 1939, Hill 1953, Schuster and Lidell 1990). In one study, a common egg-larval parasitoid of HF, *Platygaster hiemalis* Forbes, was released over a three year period in central Texas in an effort to control pest populations. During the study, HF pupal parasitism ranged from a meager 0.3 to 1.1%, and no *Platygaster hiemalis* were found after the end of the study, indicating that this parasitoid was not able to become established and thus provide ongoing control of HF populations (Rojas et al. 2000). Another study found that while both *Platygaster hiemalis* and pupal parasitoids, *Homoporus destructor* (Say) and *Eupelmus allynii* (French) were compatible with HF resistant wheat cultivars, there was no increased mortality of HF larvae due to multiple parasitism (Knutson et al. 2002).

Many types of herbivorous insects cause plants to release volatile responses that attract natural enemies and help control pest insect populations (Turlings et al. 1998, Tooker et al. 2002). However, Tooker and DeMoraes (2007) showed that HF larval feeding does not cause any indirect defensive response from wheat, and may, in part explain why natural enemies that rely on induced volatile cues are not effective on HF populations. HF also spends a relatively small amount of time exposed on leaf surfaces where general predatory insects can easily find and feed on them.

Plant Resistance

Wheat plant resistance to Hessian fly was first noted over 200 years ago (Hunter 2001). In Kansas, in 1945, it was recognized that the development of resistant wheat cultivars showed great promise in controlling HF populations (Horton et al. 1945). Today, host plant resistance is recognized by many as the most effective and cost efficient way to control HF (Ratcliff and Hatchet 1997). Currently, 34 HF resistance genes have been identified from wheat or wheat relatives (*H1-H3, h4,H5-H32 Hdic*, and *H34*) and many are currently being used or considered for use in wheat cultivars (Liu et al. 2005. Sardesai et al. 2005, Chen et al. 2009b, Li et al. 2013). However, usefulness of these cultivars is often limited by the ability of HF to rapidly overcome the resistance of specific genes once they are deployed, often within only 6-8 years, by developing new virulent biotypes (Ratcliffe et al. 1994, Ratcliffe et al. 2000, Chen et al. 2009b, Gould 1986). Furthermore, there is evidence that while these resistant cultivars provide HF protection as seedlings in the fall, resistance may break down during vernalization and wheat may be susceptible to infestation in spring. In the late spring of 2009, a wheat cultivar trial in Sedgwick County reported extensive HF damage in Duster, a cultivar that is considered resistant to HF (personal observation). This cultivar has only been available to growers since 2006, and has not been grown widely in that time. Therefore, it is not likely that it has been a long enough time for HF to develop resistance to this cultivar.

Wheat and HF demonstrate a 'gene-for-gene' interaction, a system that both benefits and hinders the development of plant resistance to this insect. Plants have resistance genes *(R*) that act as a part of the immune system and are triggered when an interaction occurs between an *R* protein and a foreign molecule produced by the attacking insect or other parasite (Nimchuck et al. 2003, Anderson and Harris 2006). In a gene-for-gene relationship, the *R* gene of the plant is matched by an avirulence (*avr*) gene of the plant parasite (Rathjen and Moffet 2003). For each *R* locus in the plant, there is a corresponding *avr* locus in the insect. Therefore, parasite adaptation to an *R* gene takes place through modifications in the corresponding *avr* gene (Harris et al. 2003). Insect-plant systems based on *R* genes are, in general, considered to be rare and therefore often overlooked (Mitchell-Olds and Bergelson 2000). However, most of these systems have been found in crop plants, including wheat, rice, maize, and barley and in interactions involving five major insect orders, Homoptera, Hemiptera, Diptera, Lepidoptera, and Coleoptera, giving this system importance from an agricultural perspective (Painter 1951, Yencho et al. 2000). From an evolutionary standpoint, *R* genes are often considered easily overcome by parasites because mutations in matching *avr* genes spread throughout the parasite population, making the parasite virulent once again and the particular *R* gene ineffective (Rausher 2001, Anderson and Harris 2006).

Interactions between grasses and HF have produced the largest number of characterized *R* genes, and these genes seem to be specific for the HF, they do not congregate with resistance to other wheat insects or diseases (Berzonsky et al. 2002, Anderson and Harris 2006). In HF populations, genotypes carrying different sets of *avr* genes are referred to as biotypes (Harris et al. 2003). Black et al. (1990) demonstrated that gene flow is not restricted among different biotypes, but large variation within biotype suggested restricted gene flow among local HF populations and therefore a high probability of inbreeding. Large amounts of inbreeding can increase the rate for new recessive *avr* mutations to become homozygous (Gould 1986).

Resistance as a plant protection method for HF has been studied extensively and is increasingly relied upon as a control measure because the reaction of the wheat's *R* genes and HFs' *avr* genes produce drastic differences in both plant and fly phenotypes (Anderson and Harris 2006). When wheat seedlings without an effective *R* gene are attacked, a compatible interaction takes place, leading to dramatic changes in wheat plant's metabolic pathways, reducing its ability to grow and develop normally (Anderson and Harris 2006, 2008,; Harris et al., 2006, 2010; Zhu et al. 2008). Wheat with an effective *R* gene, on the other hand, produces what is known as an incompatible interaction, which is not well understood, but prevents the development of nutritive cells and leads to HF larval death (Stuart et al. 2012). While outwardly, plants show no signs of HF feeding, internally at the site of HF larval attack a few epidermal cells die immediately and reactive oxygen species accumulate (Grover 1995, Harris et al. 2010, Liu et al. 2010). Epidermal and mesophyll cells adjacent to the site of attack survive with swollen mitochondria, reinforced cell walls, and an expansion of the Golgi complex-endoplasmic reticulum including small vesicles that dock at the plasma membrane (Harris et al. 2010). In addition, a granular material accumulates between the plasma membrane and outer cell walls and within the outer cell wall and are believed to be toxins as larvae feeding on these resistant plants have disrupted midgut microvilli like what would be expected from exposure to gut toxins (Giovanini et al. 2006, Subramanyam et al. 2006, Shukle et al. 2010). Studies have shown that these changes correspond with changes in plant gene expression where upregulated genes include those encoding molecules with insect toxicity, cell wall metabolism, lipid transfer proteins as well as a cultivar of lipases which may be converted to components of cuticle wax, and cell wall strengthening that prevents HF larval mouthparts from piercing cells. (De Leo et al. 2002, Dunaevsky et al. 2005, Jang et al. 2005, Liu et al. 2007, Chen 2008, Saltzmann et al. 2009, Harris et al. 2010, Kosma et al. 2010)

Of the 34 HF *R* genes currently in use, only six (*H12, H13, H18, H24, H25*, and *H26*) are still effective in southern states where HF is a consistent pest (Cambron et al. 2010). The rest have either lost efficacy or were never released commercially (Ratcliffe and Hatchett 1997). Most work in developing resistant cultivars has been done by screening seedling plants that provide resistance to HF in the fall. Currently, no published research has investigated plant resistance of more mature hard winter wheat to spring HF infestations. It has been shown that populations of HF are actively flying during the spring in Kansas, often much later in the season than previously believed (Davis et al. 2009). These populations have the ability to negatively impact wheat survival and yield and therefore mature plant resistance should be examined and delineated if possible. It is also important to quantify the impact of spring HF larvae on the plant in terms of physiology and yield.

Objectives

The objectives of this study include monitoring HF populations in Kansas by pheromone trapping to determine the validity of the historical 'fly-free date' in the fall and determine how far into the spring and summer populations are active. Pheromone trap catches will be correlated to weather events in the area to determine if and how moisture impacts adult HF emergence. In addition, this study will determine if selected commercially available wheat cultivars, advertised as having various levels of HF resistance, still provide protection to the plant, post-vernalization. Data collected will also be used to determine the physiological impact of HF infestations post-vernalization at approximately the flag leaf stage of development (Feekes 7.0 to 10.0) on selected quantifiable attributes of wheat in a susceptible cultivar.

Chapter 2 - Materials and Methods

Pheromone Lures

Pheromone-baited sticky traps were used to monitor Hessian fly (HF) populations in fields in central Kansas. When possible, fields were selected that had previous HF infestations. Pheromone trapping was conducted in several counties in Kansas: Dickinson, Reno, Saline, Sedgwick, and Smith. In each of the fields one to three sticky traps (Trécé Pherocon VI Trap, Adair, OK) were placed on the edge of fields and baited with a HF pheromone lure (PheroNet, Sweden). Traps were hung on bamboo sticks approximately 0.3m from the surface of the soil where, as shown by Anderson et al. (2012) pheromone traps caught the optimum number of male flies when they were placed within the crop canopy (Fig. 2.1). Sticky trap liners were switched out on a weekly to biweekly basis, as weather allowed, in Dickinson, Saline, Sedgwick and Smith Counties from 2009 to 2010. Trapping was conducted year around in Sedgwick County from April 12, 2011 to June 6, 2012, to determine precisely when flies are active throughout the year. Each time a trap was checked, the sticky liner and pheromone lure were removed from the trap and replaced. Except for Sedgwick County, 2011 – 2012, trapping began in late winter to spring, when wheat broke dormancy and continued until wheat was harvested. Trapping resumed in the fall, after wheat was planted and continued until adult fly emergence ended due to cold weather (when HF trap captures dropped to 0). When a sticky liner was removed from a trap, it was placed in a one gallon Ziploc® bag, labelled with the date and location, and placed in a freezer at -18°C until flies were counted. Although pheromone lures have been shown to attract only male HF (Anderson et al. 2012), a few fungus gnats and other related Dipterans would be found on the sticky traps occasionally. HF male adults were separated from other Dipterans, and counted, using a dissecting scope (6-20x) based on morphological and taxonomic characteristics as outlined *in Diagnostic Methods for Hessian Fly Mayetiola destructor* (PaDIL - Plant Biosecurity Toolbox. 2010). In addition, emerged males from a Kansas population maintained in a greenhouse were collected and used as reference specimens to aid in identification. In order to confirm that fly identification was correct, a sample of flies identified as HF males based on morphological characteristics were selected and tested for two HF-specific markers, one based on the trypsin gene, *MDP-10*, and another based on a gene encoding the salivary gland protein SSGP31-5 (Chen et al. 2014). Out of 90 flies randomly selected from sticky cards and pre-identified as HF, 88 of them were confirmed using molecular markers, indicating that flies were being correctly identified on sticky cards 98% of the time.

Weather data for Dickinson, Saline, Sedgwick locations during the time pheromone traps were deployed was collected from an Online database, Weather Underground. Data included, relative humidity(%), 24-hour high, and the 24-hour precipitation total (cm).

Hessian flies

Different biotypes or regional populations of HF used for all artificial infestations were provided by Dr. Ming-Shung Chen, USDA-ARS at Manhattan, Kansas. HF cultures were maintained at the USDA greenhouse by infesting susceptible wheat seedlings, Karl 92, with HF adults. After approximately 20 days at 21ºC, or when 80% of flies were in the pupal stage, seedling plants containing pupae were dug up, dried overnight, placed in wooden, open-top boxes that were then covered in newspaper and plastic, and placed into cold storage at -4ºC. Flies entered diapause after two weeks and did not break it for two months. After flies entered diapause they needed to remain in cold storage for at least two months. After this required period, flies could be removed, moistened, and stored at 21 ºC. In approximately 10 days, the adult flies emerged (Dendy 2009).

Greenhouse Trials

All greenhouse trials were conducted in Kansas State University greenhouses. Seeds (cultivars Fuller, Armour, and Duster), treated with Raxil® XT wettable powder fungicide (Bayer Crop Science) were vernalized at 4ºC and 100% RH for a minimum of six weeks. Seeds were saturated in 0.05g/l Terrachlor to prevent fungal growth during vernalization. Wheat seedlings were planted in Sun Gro Metro-mix 360 growing medium (Hummert International, Earth City, MO), two seedlings per five inch square Dura-pots (Hummert International, Earth City, MO). Six pots were placed into a plastic flat with no drainage holes (Hummert International, Earth City, MO) and each individual flat placed in a cage. Initially, cages were constructed of 1.3 cm PVC pipe connected to form a 0.3m by 0.6m (0.9m high) rectangle cage and covered with No SeeUm Netting (East Text Products, New York, NY). Netting was sown into square bags with one end open. Bags were placed over cage frames with the open end down and tucked under the edge of the cage. Cages were placed on thick plastic sheeting to seal the bottom of cages. These cages proved to be very susceptible to aphid entrance. After two trials had to be aborted due to significant bird cherry-oat aphid, *Rhopalosiphum padi* (Linnaeus), infestations, collapsible rearing cages (BioQuip®) were used. These cages measured 0.6m by 0.6m square (0.9m high), were fabricated with white polyester/nylon netting, mesh size ~48 x 48 on five sides and a clear vinyl sixth side. A large, protected zippered opening allowed for access to plants for infesting, watering, etc. Plants were watered daily, or as needed, by placing approximately 1cm of water into the bottom of the flats so moisture could be pulled into plants through four small holes in the bottom of the pots. Seven days after planting, each pot was treated with 1.2ml Osmocote fertilizer (Scotts Professional, North Liberty, IA). The greenhouse was maintained at 18 to 25°C and a light/dark photoperiod of 13/11. On several occasions, powdery mildew, caused by *Blumeria graminis* (DC) Speer, was observed on plants. To control the mildew without compromising the experiment, Elemental Sulfur (Greenhouse Essentials, GrowLight.com) was used in a burner (Dendy 2009). The burner was placed on a timer and operated from 11 pm to 6 am for seven consecutive days or until mildew turned brown and fell off plants.

Wheat was infested with adult HF at two growth stages, and at three HF population densities. Plants were divided into two growth stages by planting one week apart. All treatments were infested at the same time because the adult HF emerges in large numbers over a 1 -3 day period. Wheat planted on the earlier date was infested at the late flag leaf stage when the flag leaf was fully emerged to early boot stage (Feekes 9.0 to early Feekes 10.0). Plants in the later infestation group were planted one week later and therefore were one week behind in development and were infested when the second node was visible to early flag leaf stage when the flag leaf was just beginning to emerge from the whorl (Feekes 7.0 to Feekes 8.0). Each planting date was infested at three HF population densities, 0 flies (control), one female fly per tiller (low density), or three female flies per tiller (high density). Plants were infested at the flag leaf stage as this is the latest stage that HF is considered a potential pest to winter wheat in Kansas (Paulsen 1997). All 12 plants in a cage received the same treatment (same planting date and fly infestation rate). HF infestation densities were approximated based on the infestation methods used in Dr. Ming-Shun Chen's lab for testing seedling wheat for resistance (Dendy 2009). Infestation densities were calculated by counting the number of tillers in each treatment. Although plants were past tillering stages of growth and were into stem extension at the time infestations were made, there were still some small green tillers that did not ever form into culms. Therefore, when referring to infestation densities in this study they will be referred to as the number of flies/tiller rather than flies/culm. However, after plants were harvested at maturity, data were collected only from tillers that had developed into stems with spikelets and therefore data collected will be referred to on a culm basis. Using an aspirator, the proper numbers of newly emerged females were placed in cages. Two to four males were also added into cages to ensure that any unmated females had the opportunity to mate. When flies were placed in cages, they were released from approximately the bottom middle of cages. Five days after plants were infested, or when adult flies were no longer seen moving about in cages, the number of eggs on each plant were counted using a small hand lens.

Due to limited greenhouse space, two separate trials had to be conducted for the cultivars Fuller and Duster. For each cultivar one trial began in the spring and one began in the fall. No trials were conducted in the heat of the summer or coldest parts of the winter when greenhouse temperatures could not be maintained at the desired temperatures. For the cultivar Armour an additional greenhouse was used and therefore the entire experiment was done at one time, during the fall.

Once plants reached physiological maturity, they were removed from pots, including the base of the plant, and individual plants were placed in one gallon Ziploc® bags. Plants were immediately frozen and maintained at -18°C until they could be analyzed and data collected. Collected data consisted of culm height (cm), measured from the base of the stem (not including roots) to the highest spikelet, (flowers, each of which may contain multiple florets which become seeds) not including the awn, number of spikelets/spike (panicle/head), number of seeds/spikelet, weight of seeds/ spike (grams), number of HF flaxseeds/culm, and number of culms/plant. A completely randomized design was utilized for all trials. Culm data were averaged over the plant. Data were analyzed using SAS 9.3 for analysis of variance (ANOVA) using the PROC

GLM procedure with HF infestation level (0, 1 fly/tiller, or 3 flies/tiller) as the main effect and blocked on greenhouse trial. Means were separated using LSmeans. For the HF susceptible cultivar, Fuller, data were further analyzed using the PROC GLM procedure with the number of HF flaxseeds present in each culm (0, 1-5, 6-15, 16-30 and 31-86) as the main effect.

Field Trials

Wheat cultivars included in the county cultivar trials in Saline (2010-13) and Sedgwick (2010-2012) were utilized to conduct HF infestation trials. In Saline County, Fuller, a HF susceptible cultivar, was infested every year 2010-2013. Armour, a cultivar with intermediate resistance was infested in 2010, 2012, and 2013. In 2011, the cultivar Post Rock was inadvertently infested instead of Armour (See results – Appendix A). In 2013, Duster, a cultivar considered to be highly resistant, was available for infestation at both locations in Saline County for the first time. In Sedgwick County, Fuller (susceptible) and Duster (resistant) were infested each year 2010-2012. In 2010, a mistake in the plot map led to the inadvertent infestation of two plots, one planted with the cultivar Post Rock and one with the cultivar 'TAM 204' (See results –Appendix A). In 2013, no cultivar trials were available in Sedgwick County therefore cages were placed at two separate locations in Saline County. Each year, when wheat reached the flag leaf stage, (Feekes 7.0 to Feekes 9.0), cages were constructed and plants artificially infested. In 2010, field cages consisted of 1.3cm inch PVC pipe duct taped together to form a 0.3m by 0.46m rectangle. This was placed on the soil surface around the wheat plants and No SeeUm Netting (East Text Products, New York, NY) was gently laid over the top of plants and tucked under the edge of the PVC. Netting was held in place using wire flags at each corner and one along each side of the cage, effectively creating a cage that would allow as much air movement and sunlight as possible to reach plants, but would also remain flexible, causing no damage to wheat (Fig. 2.2). For the 2011-2013 field seasons cages that were initially designed for use in the greenhouse were used in the field. These cages were constructed of 1.3 cm PVC pipe connected to form a 0.3m by 0.6m (0.9m high) rectangle cage and covered with No SeeUm Netting (East Text Products, New York, NY). Netting was sown into square bags with one end open. Bags were placed over the cage frames with the open end down and tucked under the edge of the cage. At each field location four cages were randomly placed in each selected cultivar. Two cages were infested with HF and two cages were not infested to serve as the control. Cages selected to be infested, were infested using HFs reared in the lab. After cages were constructed, the numbers of tillers present were counted and enough female flies were released to allow for one adult female fly/wheat tiller. Flies were released in each cage using an aspirator, and two to four males were added to ensure any unmated females had the opportunity to mate. Once cages were infested, soil was pushed up along the PVC to ensure that cages were sealed and no gaps remained between cages and the soil surface. Netting was left in place for three to five days, long enough to ensure that all females had oviposited and completed their life cycle, but not long enough to impact the growth of wheat plants.

Once plants reached physiological maturity, they were removed from the plots, at the base of the plant, and groups of culms were placed in one gallon Ziploc® bags. All culms from each individual cage were then placed in a larger bag and transported back to the lab where they were stored at -18°C until data could be recorded. In 2013, plants were too tall to place in one gallon bags, so all culms from one plot were placed in a large 30 gallon Hefty Cinch Sak® trash bag with another 30 gallon bag placed over the top to prevent any seeds or plant parts from falling out of the top. Collected data consisted of culm height (cm), measured from the base of the stem (not including roots) to the highest spikelet, (flowers, each of which may contain multiple florets which become seeds) not including the awn, number of spikelets/spike (panicle/head), number of seeds/spikelet, weight of seeds/spikelet (grams), and number of HF flaxseeds/culm. As it was difficult to clearly determine what constituted an individual plant when harvesting the wheat, data were analyzed on a culm basis and we were not able to determine the number of culms/plant as in the greenhouse. Data were analyzed using SAS 9.3 for analysis of variance (ANOVA) using the PROC GLM procedure with HF infestation level (infested or non-infested) as the main effect and blocked by field location and year. A culm was considered infested if a flaxseed was present at the time of data collection. Means were separated using LSmeans.

Figures and Tables

Figure 2.1 Pheromone trap placed in a field in Dickinson County, Kansas.

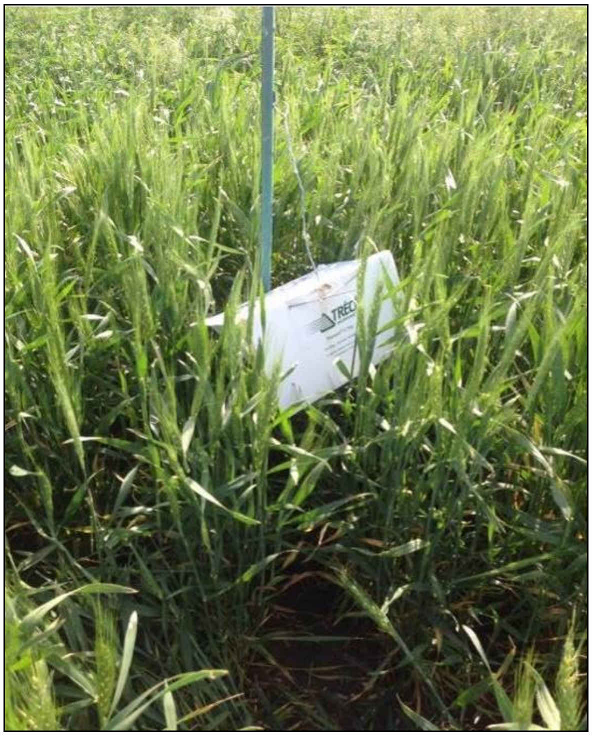

Figure 2.2 Field cages placed in the field for artificial Hessian fly infestations. Photo on the left shows type of cage used in 2010. Photo of the right shows type of cage used in 2011-2013.

Chapter 3 - Results – Pheromone Trapping

Hessian fly-free date

Previous studies in the Mid-West and Great Plains have reported that Hessian flies (HF) emerge in two main broods or have two generations per year; one in the spring and one in the fall (Byers and Gallun 1971). Specifically in Kansas, Dean and McCulloch (1915) reported the spring brood emerges in April and fall brood between late August and October, most commonly during late September. However, this same publication mentions four and five broods reported in 1908 and 1914. While dry, cool weather is reported to lengthen the HF life cycle and moist, warm weather shortens it, very little research has been done in Kansas since the early 1900's to better understand HF activity in the field. This is largely due to the fact HF is a very difficult fly to monitor in the field. Adults are small and short-lived, the eggs are very small and may be easily mistaken for leaf rust, and larvae and pupae spend most of their time hidden in the leaf sheath where sampling for them requires destruction of the plant. Synthetic sex pheromones have been used to monitor a number of important crop pests, allowing for a better understanding of their biology and leading to the development of better control measures. Interest in HF sex pheromone began in 1922 when it was noted that in a field setting, caged females could attract male flies from at least 3m away (Cartwright 1922). Then, in 1991, a major component of the pheromone was identified, but its failure to attract males in the field led to many years of research to identify four other important components (Foster et al. 1991b). Finally, the five-component blend was successfully produced and was shown to attract males in the laboratory, small plots, and in Kansas wheat fields (Andersson et al. 2009). With HF sex pheromone commercially available it became feasible to re-examine the historically established HF-free date. In addition, much needed work could be done to confirm or disprove the previously noted two main broods in Kansas and, if possible, to determine what environmental factors may play a role in when these main and supplementary broods emerge.

In all Kansas counties where fall HF pheromone trapping was conducted, flies were captured at least one month after the historical HF-Free Date, thus indicating that the historically used fly-free date is no longer accurate and should be referred to as the Best Pest Management Date (Table 3.1). In addition, pheromone trapping in both fall and spring showed the HF in Kansas does not emerge in two major broods as previously thought. Trapping indicates these flies are constantly emerging, at least in small numbers, and have numerous small emergence spikes throughout the growing season, into summer, and again during the fall (Figs. 3.1 – 3.4 and Appendix B). Presumably these flies are mating and laying eggs throughout the spring, summer, fall and into winter. Additionally, adults may emerge from wheat stubble remaining in the field and therefore, may become established in volunteer wheat if left to grow throughout the summer. This may aid in building up local populations of HF and, if environmental conditions are favorable, these flies may cause significant plant death in the fall or yield loss the following spring.

Hessian fly emergence related to weather events

Historically, HF populations have reportedly been more significant in wet years with moderate temperatures and less in warm to hot, dry years (Dean and McCulloch 1915, Criddle 1917). The temperature maximums and minimums for the development of

HF has been well documented, but there is little information relating how moisture events may impact the emergence of HF adults from the pupal stage (Foster and Taylor 1974). Pike and Antonelli (1981) report that, in Washington, HF may emerge from flaxseeds approximately 12 days after a rain of 1.3cm or more. However, this appears to be based on observation with no empirical evidence to support it. Results from this study do not show a strong correlation between moisture events, either rain or high humidity, and emergence of adult HF (Figs. 3.2-3.4 and Appendix B). Trapping conducted in Sedgwick County throughout the spring 2011 and into spring 2012 best illustrates this as traps were checked on a regular basis throughout the year (Figs. 3.2-3.4). While these results suggest that further studies need to be conducted to investigate what environmental factors, if any, may be influencing the emergence of HF, it was beyond the scope of the current research. Currently, field studies are being conducted in Reno and Dickinson counties in Kansas which are attempting to delineate, more specifically, how moisture events may impact HF emergence.

Figures and Tables

Figure 3.1 Pheromone trap data for the spring seasons from 2009 to 2012 in Sedgwick Co., KS, showing continual emergence of Hessian flies. Each symbol represents a trap (location and year). Trap capture of 996 flies at location 2 on June 2, 2009 not shown so that data could be observed better.

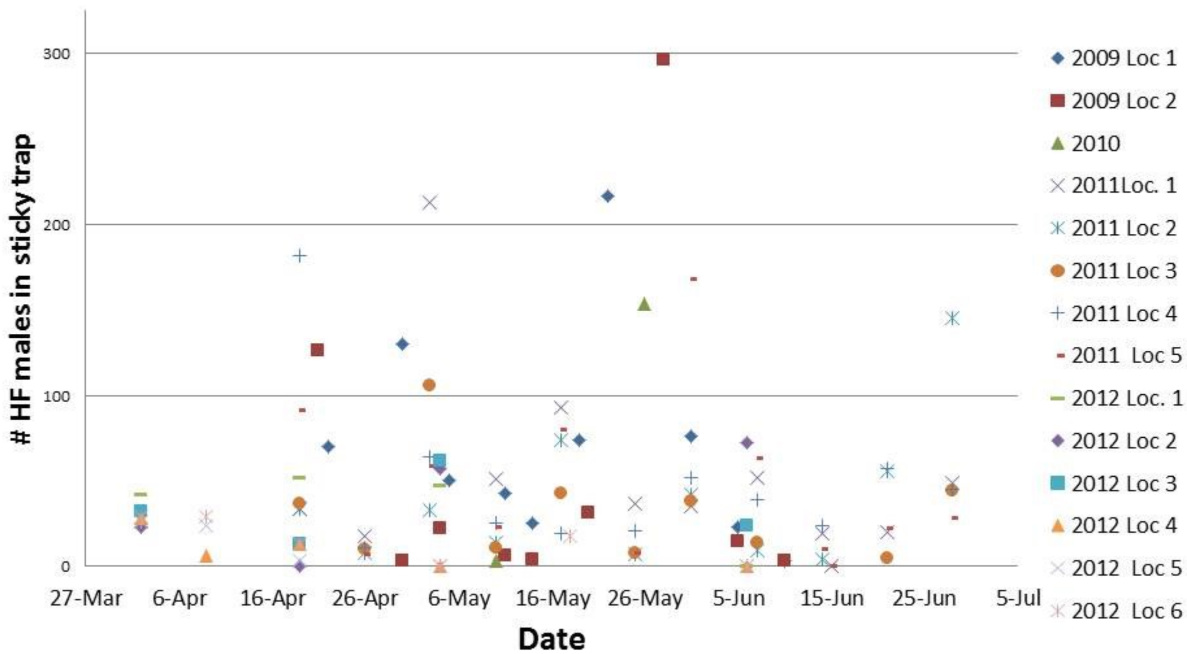

Figure 3.2 Pheromone trap data for Sedgwick County - 16 April to 31 August, 2011. Each trap catch represents the total of 5 traps. Blue line represents 24-hour precipitation totals (cm), green line represents the high relative humidity recorded during that 24-hour period (%). Wheat was harvested 3 July, 2011.

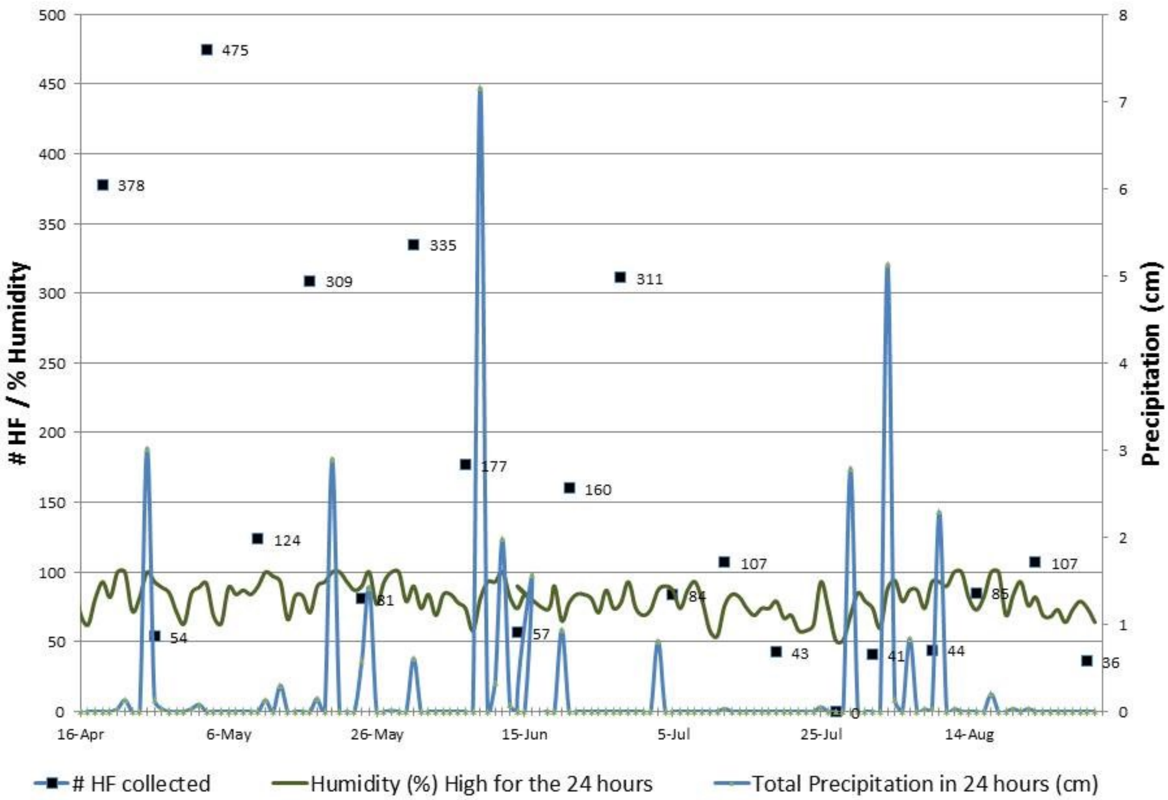

Figure 3.3 Pheromone trap data for Sedgwick County - 1 September to 3 November, 2011 (4 November to 12 March are not shown as there were no Hessian flies caught on traps during that time). Each trap catch represents the total of 5 traps. Blue line represents 24-hour precipitation totals (cm), green line represents the high relative humidity recorded during that 24-hour period (%).

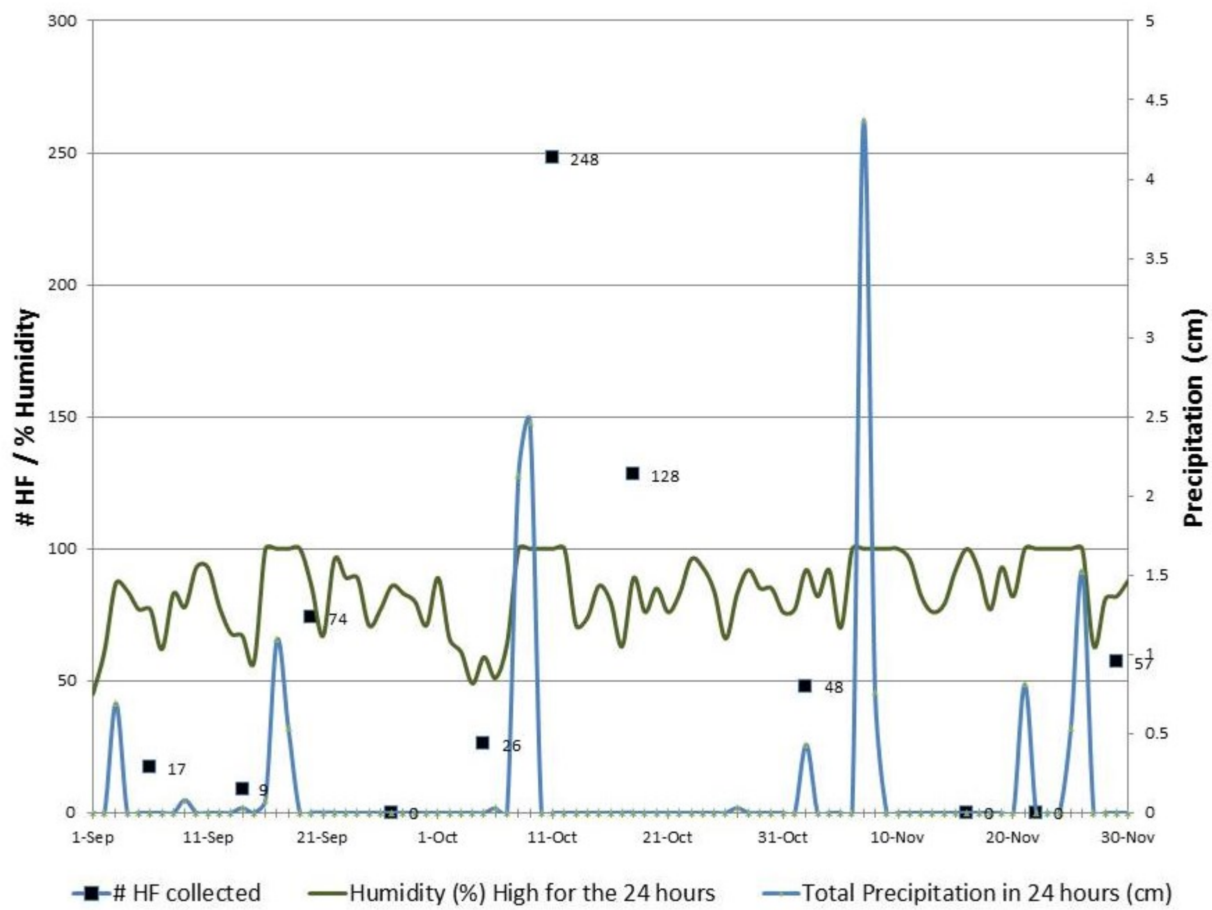

Figure 3.4 Pheromone trap data for Sedgwick County - 12 March to 20 June, 2012 (4 November to 12 March are not shown as there were no Hessian flies caught on traps during that time). Each trap catch represents the total of 5 traps. Blue line represents 24-hour precipitation totals (cm), green line represents the high relative humidity recorded during that 24-hour period (%). Wheat was harvested 30 May, 2012.

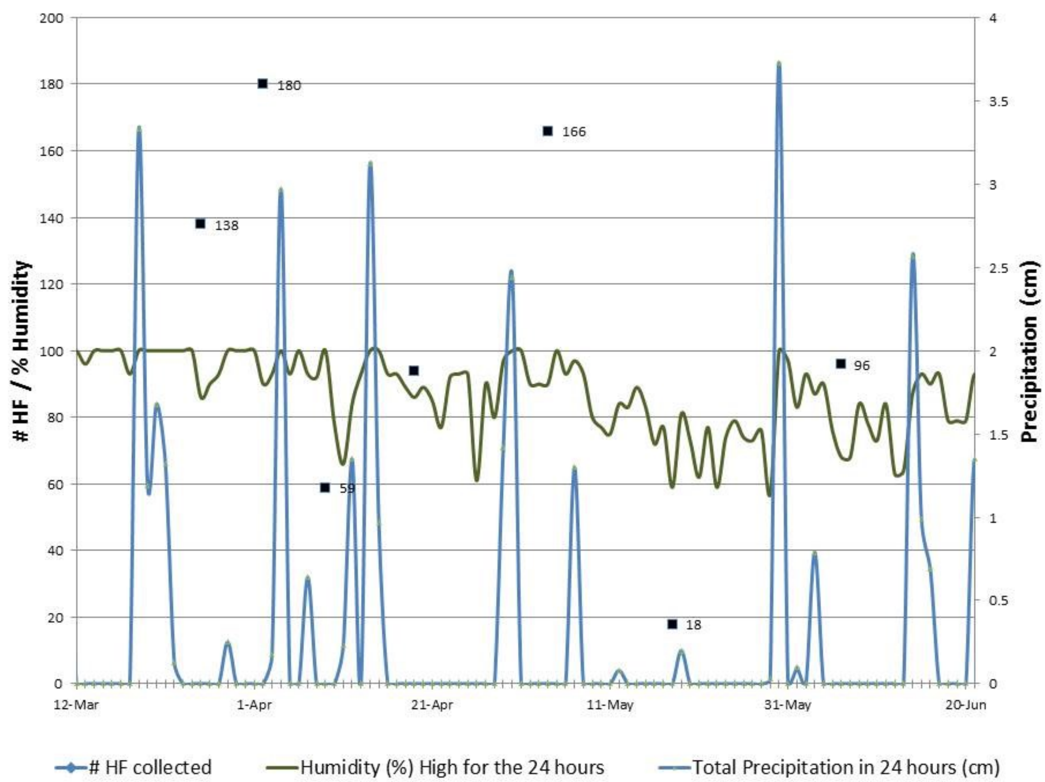

Table 3.1 Showing the latest date that a male Hessian fly was caught in a pheromone trap. Data collected from 2009 to 2011, from selected counties in north central, central, and south central areas of Kansas, as these areas have had periodic problems with Hessian flies since the 1980's .

County	Historical Fly-Free Date	Latest capture date of HF on a sticky card
Dickinson	6 October	6 December
Saline	6 October	1 December
Sedgwick	10 October	9 December
Smith	29 September	13 November

Chapter 4 - Results - Greenhouse Trials

Egg and flaxseed counts

Five days after cages had been infested with adult, Hessian fly (HF) females, egg counts were conducted to ensure that plants in the control groups did not contain any eggs and females were successfully laying eggs on plants in the treatment groups. Results of these counts confirmed that none of the female flies were able to fly out of the cages they were placed in and into control cages to infest plants (Fig. 4.1). Conversely, eggs were successfully laid on all plants in the treatment groups. There was a large variation in the mean number of eggs laid on each plant for the different cultivars with Fuller having the fewest number of eggs/plant for both the low infestation rate of 1 fly/tiller and the high infestation rate of 3 flies/tiller. Average number of eggs/plant increased dramatically when adult fly densities went from 1 fly/tiller to 3 flies/tiller, 5.9 to 61.0 eggs/plant for Fuller and 18.6 to 122.8 eggs/plant for Armour (Fig. 4.1). Egg densities were larger than expected for the infestation level of 1 fly/tiller in Duster but still nearly doubled from 55.7 to 92.3 eggs/plant when the infestation density was increased (Fig. 4.1) In greenhouse resistance screening trials conducted on seedlings, plants are typically infested with adult HF around the 1.5 to 2 leaf stage and egg densities are approximately 5 to 8 eggs/plant (Chen et al. 2009b, Dendy 2009). While it is difficult to compare egg densities from seedling trials to those we conducted on plants in the flag leaf stage (Feekes 7-10), there was an average of 3-5 tillers/plant, with varying numbers of leaves/tiller, but averaging roughly 4 leaves/tiller. Therefore, the egg densities, when plants were infested with 1 fly/tiller, in this study were typically lower than those used in seedling resistance screening. However, the higher infestation density of 3 flies/tiller used in this study produced similar egg densities to those used in resistance screening. Due to the difficultly of accurately sampling HF eggs in the field, no data were located to indicate what egg densities may be in a naturally occurring field situation. At experiment termination and after plants were harvested, the number of HF flaxseeds (pupae)/plant was recorded. In the HF susceptible cultivar, Fuller, there was an average of 3.5 HF flaxseeds /culm or 17.5/plant on plants infested at 1 fly/tiller, and an average of 9.7 flaxseeds/culm or 61.7/plant when infested with 3 flies/tiller (Fig. 4.1). In a 9-year study conducted in Georgia on HF susceptible cultivars of soft red winter wheat, the number of flaxseeds/culm in the spring ranged from 0-10, with the average being 1.97 (Buntin 1999). It is difficult to compare this greenhouse work with field work conducted in Georgia on a different class of wheat. Additionally, HF larvae and pupae in the field face a number of environmental difficulties leading to mortality that those in the greenhouse do not. However, this comparison does indicate that infestations on the susceptible cultivar, Fuller, in the greenhouse met and exceeded what one may expect to find in the field. In Armour, a cultivar with intermediate resistance, there was an average of 0.2 HF flaxseeds/culm or 1.1/plant in the plants infested at 3 flies/tiller but there were no flaxseeds found in culms and plants infested at 1fly/tiller (Fig. 4.1). In Duster, a cultivar considered highly resistant to HF, there were no flaxseeds recovered from plants infested with 1fly/tiller and an average of 0.04 HF flaxseeds/culm or 0.1/plant in the plants infested at 3 flies/tiller (Fig. 4.1).

The average number of flaxseeds/plant was then compared to the original egg counts for each cultivar at each infestation level (Fig. 4.1). For the infestation level of 1 fly/tiller in Fuller, an average of 17.5 flaxseeds was found/plant, while an average of only 5.9 eggs/plant was recorded (Fig. 4.1). This suggests that a number of eggs may have been overlooked. When egg counts were made, great care was taken not to injure plants, therefore it is reasonable to assume that some eggs deposited on leaves close to the stem may not have been counted. Regardless, these egg and flaxseed counts do demonstrate that eggs were viable and survived well on the susceptible cultivar, Fuller. On the other hand, although eggs were deposited on the intermediately resistant cultivar, Armour, and the resistant cultivar, Duster, very few larvae survived to develop into the pupal stage. This is not surprising as previous studies have shown that plant resistance to HF takes place as early instar larvae begin to feed (Harris et al. 2010, Stuart et al. 2012).

After plants were harvested and data were collected, comparisons were made within each cultivar between culms at each infestation level. The different cultivars were not compared with each other because there is a known variation among different cultivars due to their pedigrees and not related to HF larval feeding.

Culm height

In the susceptible cultivar, Fuller, there was a significant reduction in the average culm height due to HF larval feeding. Plants infested with 1 fly/tiller lost an average of 7.4cm/culm and at the higher infestation rate of 3 flies/tiller lost an average of 11.2cm/culm compared to the control (Fig. 4.2). In the intermediately resistant cultivar, Armour, there was a slight height decrease between non-infested plants and those infested at 1 or 3 flies/tiller, but it was not significant (Fig. 4.2). In the resistant cultivar, Duster, there was a significant decrease in culm height between plants that were not infested and those infested with 1 fly/tiller. However, at infestations of 3 flies/tiller the culm height was not significantly different from non-infested plants or plants infested with 1 fly/tiller. There were no significant differences in culm height related to the planting date for any of the cultivars, meaning whether the plants were infested at the late early flag leaf stage (Feekes 7-8) or the late flag leaf stage (Feekes 9-10) and fly infestation level (Table 4.1)

Number of spikelets per spike

In the susceptible cultivar Fuller, the number of spikelets (flowers, each of which may contain multiple florets which become seeds)/spike (panicle/head) decreased significantly by almost 1 spikelet/spike between non-infested plants and those that were infested with 3 flies/tiller. However, there was not significant decrease in the number of spikelets/spike between non-infested plants and plants infested with 1 fly/tiller or between plants infested at 1 and 3 flies/tiller (Fig. 4.3). There were significant interactions between the HF infestation level and which plant growth stage the plants were infested (Table 4.1). In non-infested plants and those infested with 1 fly/tiller, there were significantly more spikelets/spike when they were infested at the early flag leaf stage (Feekes 7-8). Conversely, in plants infested at 3 flies/tiller, there were significantly more spikelets/spike in plants infested at the late flag leaf stage, (Feekes 9-10). There was no significant difference in the number of spikelets/spike for any of the treatments in Armour, although there was a very slight trend for the number of spikelets to decrease as infestation level increased (Fig. 4.3). There were no significant interactions between HF infestation level and plant growth stage infested (Table 4.1).

In Duster, there was a significant decrease of one spikelet/spike between non-infested plants and plants infested with 1 fly/tiller. However, when plants were infested with 3 flies/tiller there was no significant difference from the non-infested plants (Fig. 4.3). There were significant interactions between the infestation levels and the growth stage of plant infested (Table 4.1). In both the non-infested plants and those infested with 1 fly/tiller plants infested at the early flag leaf stage (Feekes 7-8) had significantly fewer spikelets/spike than plants infested a week later (Table 4.1). However, plants infested with 3 flies/tiller did not show any significant difference in the number of spikelets/spike when infested at either plant growth stage.

Number of seeds per spikelet

The number of seeds/spikelet decreased significantly in the susceptible cultivar, Fuller, when plants were infested at either 1 or 3 flies/tiller, but did not decrease significantly between the two infestation levels (Fig. 4.4). There was no significant difference in the number of seeds/spikelet between the two plant growth stages for the non-infested and lower infestation level (Table 4.1). When plants were infested with 3 flies/tiller, plants infested at the early flag leaf stage (Feekes 7-8) had significantly fewer seeds/spikelet than those infested at the flag leaf stage (Feekes 9-10). In Armour, a cultivar with intermediate resistance, there was a trend for fewer seeds/spikelet as the HF infestation level increased, but it was not significant (Fig. 4.4). There were no significant differences between plants infested at either plant growth stage (Table 4.1). In the resistant cultivar, Duster, no significant differences in the number of seeds were observed for any of the fly infestation levels nor where there any significant differences between plants infested at either plant growth stage (Fig. 4.4, Table 4.1).

Weight of seeds per spike

In Fuller, the weight of seeds/spike decreased significantly, over 0.1g/spike when plants were infested with either 1 or 3 flies/tiller, but did not decrease significantly between the two infestation levels (Fig. 4.5). There was no significant difference in the weight of seeds/spike between plants infested at either growth stage with 1 fly/tiller vs. the non-infested plants (Table 4.1). However, when plants were infested with 3 flies/tiller there was a significant difference between infestation dates, with plants infested at the early flag leaf stage (Feekes7-8) having seed weights of half of those plants infested at the late flag leaf stage(Feekes 9-10).

The weight of seeds/spike in Armour did not decrease significantly between the control and plants infested with 1 fly/tiller (Fig. 4.5). However, the seed weight decreased significantly, over 0.1gm/spike, when plants were infested with 3 flies/tiller. There were no significant differences between infestation levels at either of the plant growth stages infested (Table 4.1). For the resistant cultivar, Duster, the weight of seeds/spike actually increased significantly with the higher infestation levels of 3 flies/tiller (Fig. 4.5). There were no significant differences in seed weights between the control and plants infested with 1 fly/tiller. There was a significant difference in seed weight in non-infested plants where plants planted 1 week earlier (would be infested at Feekes 9-10) had significantly heavier seeds than those planted 1 week later (would be infested at Feekes 7-8) (Table 4.1). This difference was not found in plants infested with 1 fly/tiller and in plants infested with 3 flies/tiller, plants infested at the early flag leaf stage (Feekes 7-8) had heavier seeds than those infested at the late flag leaf stage (Feekes 9-10).

Number of culms per plant

In Fuller, the number of culms/plant increased significantly by approximately 2 stems/plant when infested with 1 or 3 flies/tiller, although a number of these culms were small and did not produce seed or produced very little seed (Fig. 4.6). There was not a significant difference in the number of culms between infestations of 1 and 3 flies/tiller. There were no significant differences in the number of culms based on the developmental stage the wheat was infested at in the control or infestation of 1 fly/tiller (Table 4.1). However, when infested with 3 flies/tiller, the number of culms/plant decreased significantly if infested at the early flag leaf stage (Feekes 7-8) vs. the late flag leaf stage (Feekes 9-10). In Armour, there was a decreasing trend in the number of culms/plant as the HF levels increased, but it was not significant (Fig. 4.6). There was no significant difference in the number of culms/plant based on the growth stage in which plants were infested (Table 4.1). In Duster, there were no significant differences in the number of culms/plant based on infestation level (Fig. 4.6). In non-infested plants, there were significantly more culms/plant in plants that were planted one week earlier (would be infested at Feekes 9-10) (Table 4.1). But there were no significant differences based on planting and infesting date related to infestation levels of 1 or 3 flies/tiller.

With Fuller and Duster, each greenhouse trial was conducted twice, one beginning in the spring and one beginning in the fall. No trials were conducted in the heat of the summer or coldest parts of the winter when greenhouse temperatures could not be well maintained at the desired temperatures. Regardless, there was a significant difference in the plants grown in the greenhouse based on which greenhouse trial they belonged to. Plants in trials that began in the spring and went into summer tended to be more robust overall. As shown in Table 4.2, culm height, number of spikelets/spike, and seed weight/spike are significantly greater in both Fuller and Duster in trials initiated in the spring than in fall initiated trials. There was no significant difference in number of seeds/spikelet in Duster; however, Fuller had more seeds/spike in the spring trial. There was no difference in the number of culms/plant for Fuller however, for Duster, the spring trial had significantly fewer culms/plant. Greenhouse trials for Armour were conducted in one large trial during the fall, therefore there are no differences based on which greenhouse trial they belonged to.

Physiological impact of Hessian fly on Fuller

For Fuller, a HF susceptible cultivar, culms were separated into five groups based on how many HF flaxseeds were present (0, 1-5, 6-15, 16-30 or 31-86). It was found that the presence of 1 to 5 flaxseeds significantly reduced culm height compared to non-infested plants (Fig. 4.7). However, up to 15 flaxseeds did not cause any further significant loss in culm height. When 16 – 30 and 31 - 86 flaxseeds were collected from a single culm, plant height dropped significantly compared to culms with 1-15 and 6-15 flaxseeds.

The number of spikelets/spike did not significantly decrease significantly from non-infested plants until there were 6 or more flaxseeds present/culm (Fig. 4.8). Although these culms had significantly fewer spikelets than the non-infested culms, they did not have significantly fewer spikelets than plants containing 1-5 flaxseeds. The number of spikelets did not significantly decrease further until there were at least 31 flaxseeds/culm.

The number of seeds/spikelet decreased significantly with the presence of 1-5 flaxseeds, but then was not significantly different from non-infested plants or those with 1-5 flaxseeds when 6-15 flaxseeds were present (Fig. 4.9). As with other variables, it took a minimum of 16 flaxseeds/culm to further reduce the number of seeds/spikelet.

There was a significant loss in seed weight/culm if at least 1 flaxseed was present (Fig. 4.10). Seed weight continued to decrease as the number of flaxseeds increased and, in fact, with the presence of 31 or more flaxseeds there was very little seed to harvest with many heads containing no measureable seed. All variables, other than the number of spikelets/spike, a significant reduction is found when at least one flaxseed is present.

Figures and Tables

Figure 4.1 Average number of Hessian fly eggs/plant, recorded five days post-infestation and average number of flaxseeds/pupae per plant, recorded post-harvest.

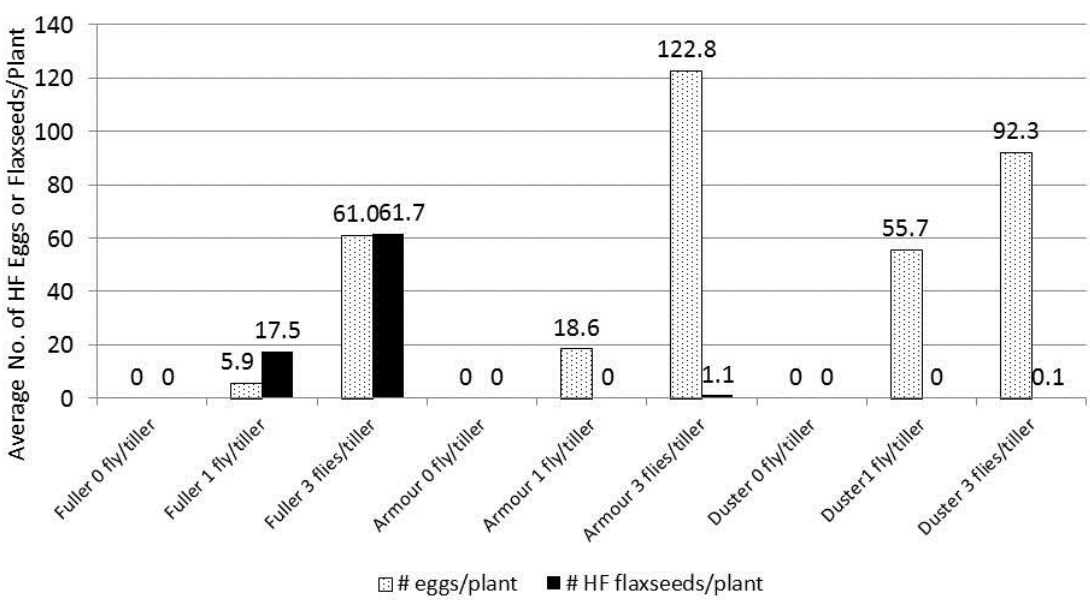

Figure 4.2 Greenhouse. Average culm height (cm). Treatment means within the same group (cultivar) with the same letters are not significantly different (*P* ≤ 0.05*)*.

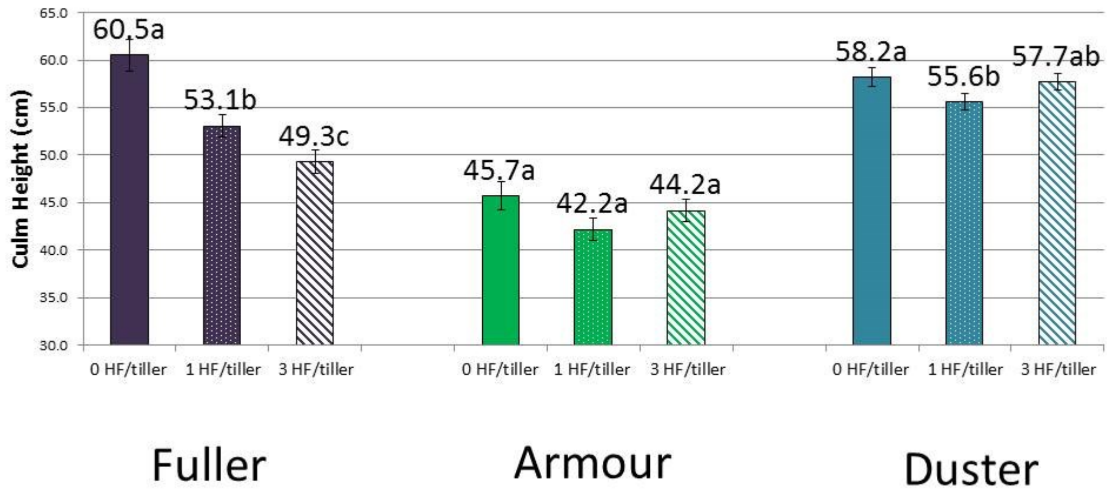

Figure 4.3 Greenhouse. Average number of spikelets/spike. Treatment means within the same group (cultivar) with the same letters are not significantly different (*P* ≤ 0.05*)*.

Figure 4.4 Greenhouse. Average number of seeds/spikelet. Treatment means within the same group (cultivar) with the same letters are not significantly different ($P \leq 0.05$).

Figure 4.5 Greenhouse. Average weight (grams) of seeds/spike. Treatment means within the same group (cultivar) with the same letters are not significantly different ($P \leq 0.05$).

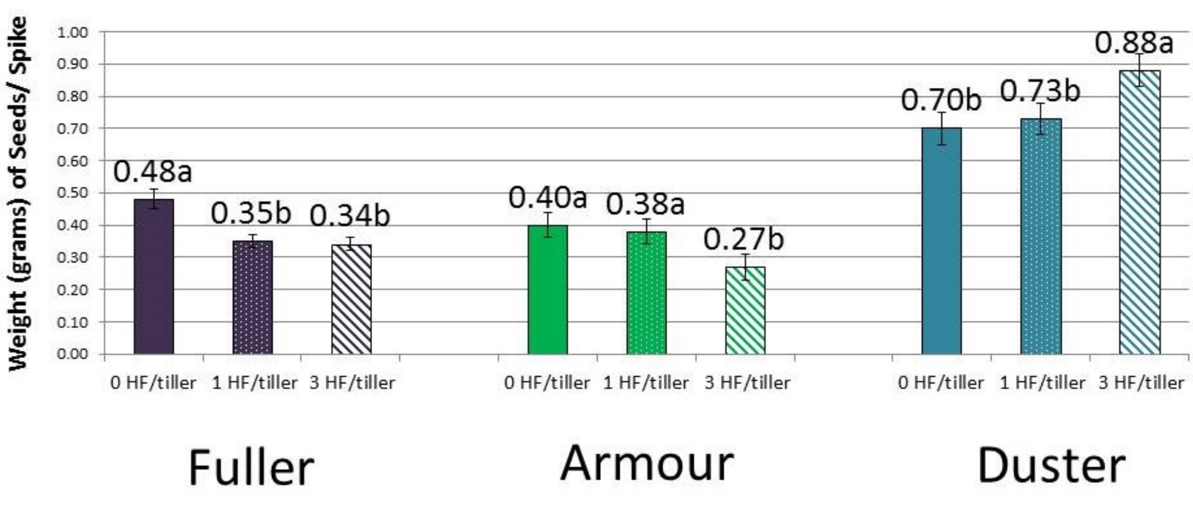

Figure 4.6 Greenhouse. Average number of culms/plant. Treatment means within the same group (cultivar) with the same letters are not significantly different (*P* ≤ 0.05*)*.

Figure 4.7 Average culm height (cm) for the cultivar Fuller with 0, 1-5, 6-15, 16-30, or 31-86 flaxseeds/culm at harvest. Treatment means with the same letters are not significantly different (*P* ≤ 0.05*)*.

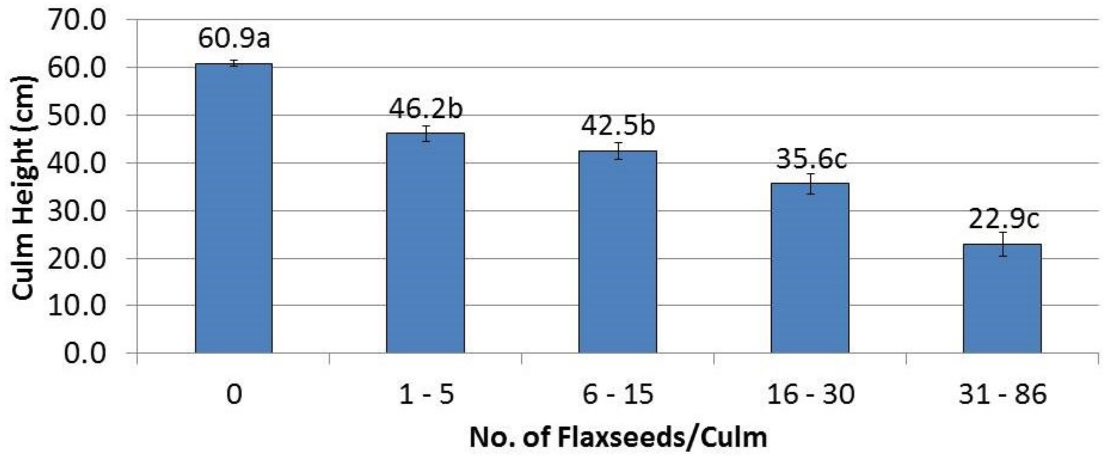

Figure 4.8 Average number of spikelets/spike for the cultivar Fuller with 0, 1-5, 6-15, 16-30, or 31-86 flaxseeds/culm at harvest. Treatment means with the same letters are not significantly different (*P* ≤ 0.05*)*.

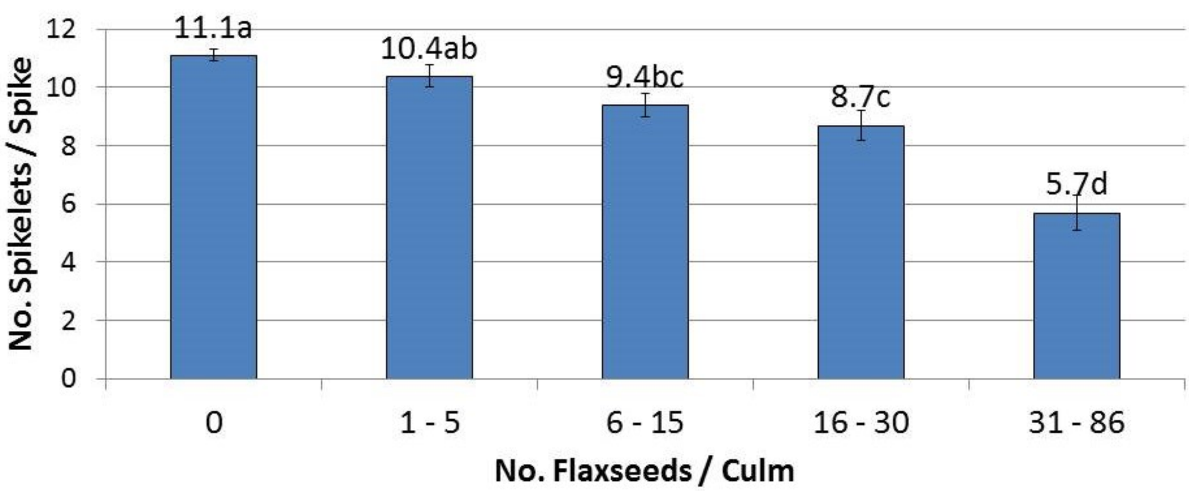

Figure 4.9 Average number of seeds/spikelet for the cultivar Fuller with 0, 1-5, 6-15, 16-30, or 31-86 flaxseeds/culm at harvest. Treatment means with the same letters are not significantly different (*P* ≤ 0.05*)*.

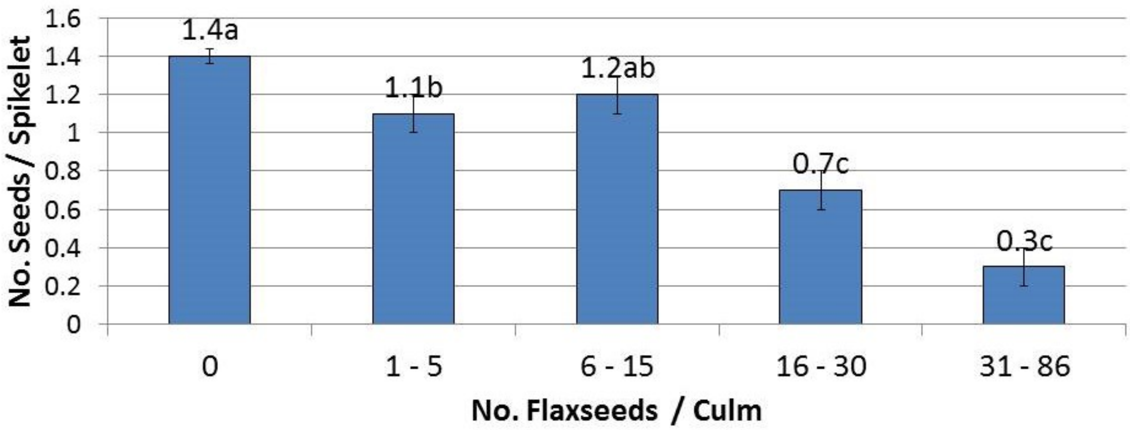

Figure 4.10 Average weight of seeds/spike (grams) for the cultivar Fuller with 0, 1-5, 6-15, 16-30, or 31-86 flaxseeds/culm at harvest. Treatment means with the same letters are not significantly different (*P* ≤ 0.05*)*.

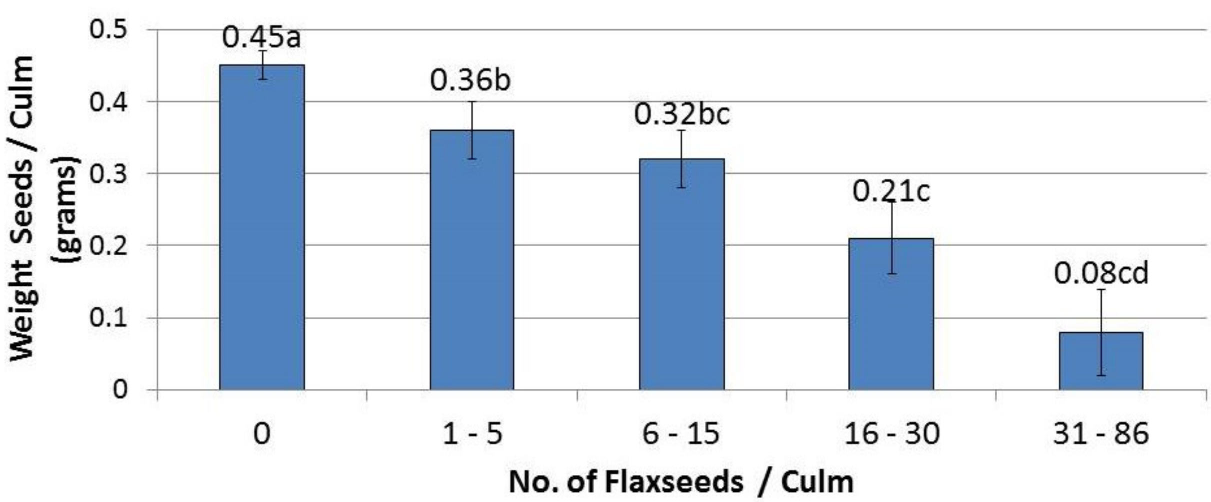

Table 4.1 Greenhouse. Wheat cultivar and Hessian fly infestation level interaction with selected plant growth stage infested. Treatment means within the same group (cultivar and infestation level) with the same letters are not significantly different ($P \leq 0.05$).

Wheat Cultivar and Hessian Fly infestation level	Wheat growth stage	Culm Height (cm)	Spikelets/Spike	Seeds/Spikelet	Seed Weight/Spike (grams)	Culms/Plant
Fuller - 0 flies/tiller	Feekes 7-8	62.1 ± 2.70a	**11.6 ± 0.57a**	1.6 ± 0.13a	0.51 ± 0.05a	3.9 ± 0.70a
Fuller - 0 flies/tiller	Feekes 9-10	58.8 ± 1.85a	**9.9 ± 0.39b**	1.6 ± 0.09a	0.44 ± 0.03a	4.4 ± 0.48a
Fuller - 1 fly/tiller	Feekes 7-8	55.1 ± 1.52a	**11.2 ± 0.32a**	1.3 ± 0.08a	0.34 ± 0.03a	5.5 ± 0.40a
Fuller - 1 fly/tiller	Feekes 9-10	50.8 ± 1.85a	**9.4 ±0.39b**	1.1 ± 0.09a	0.36 ± 0.03a	6.5 ± 0.47a
Fuller - 3 flies/tiller	Feekes 7-8	47.8 ± 1.85a	**9.3 ± 0.39b**	**0.9 ± 0.09b**	**0.23 ± 0.03b**	**4.8 ± 0.48b**
Fuller - 3 flies/tiller	Feekes 9-10	50.8 ± 1.85a	**10.4 ± 0.39a**	**1.2 ± 0.09a**	**0.46 ± 0.03a**	**7.0 ± 0.47a**
Armour - 0 flies/tiller	Feekes 7-8	44.9 ± 2.11a	10.5 ± 0.56a	2.0 ± 0.13a	0.41 ± 0.05a	7.0 ± 0.93a
Armour - 0 flies/tiller	Feekes 9-10	46.5 ± 2.29a	10.7 ± 0.61a	2.1 ± 0.15a	0.39 ± 0.05a	6.7 ± 0.85a
Armour - 1 fly/tiller	Feekes 7-8	42.2 ± 2.29a	10.6 ± 0.56a	1.9 ± 0.15a	0.40 ± 0.05a	6.4 ± 0.93a
Armour- 1 fly/tiller	Feekes 9-10	42.2 ± 2.11a	10.4 ± 0.61a	1.8 ± 0.13a	0.36 ± 0.05a	5.2 ± 0.85a
Armour - 3 flies/tiller	Feekes 7-8	43.4 ± 2.11a	10.5 ± 0.61a	1.8 ± 0.13a	0.28 ± 0.05a	6.0 ± 0.85a
Armour - 3 flies/tiller	Feekes 9-10	45.0 ± 2.29a	10.1 ± 0.61a	1.9 ± 0.15a	0.26 ± 0.05a	5.2 ± 0.93a
Duster - 0 flies/tiller	Feekes 7-8	56.5 ± 1.63a	**12.8 ± 0.44b**	2.1 ± 0.18a	**0.58 ± 0.07b**	**5.0 ± 0.28a**
Duster - 0 flies/tiller	Feekes 9-10	59.7 ± 1.01a	**14.3 ± 0.28a**	2.3 ± 0.12a	**0.83 ± 0.05a**	**4.1 ± 0.18b**
Duster - 1 fly/tiller	Feekes 7-8	53.4 ± 1.57a	**11.8 ± 0.42b**	2.2 ± 0.17a	0.69 ± 0.07a	4.2 ± 0.27a
Duster- 1 fly/tiller	Feekes 9-10	57.2 ± 1.01a	**13.4 ± 0.28a**	2.0 ± 0.11a	0.77 ±0.04a	4.8 ± 0.18a
Duster - 3 flies/tiller	Feekes 7-8	58.2± 1.57a	13.8 ± 0.28a	2.3 ± 0.17a	**0.96 ± 0.07a**	4.6 ± 0.27a
Duster- 3 flies/tiller	Feekes 9-10	57.4 ± 1.01a	13.8 ± 0.42a	2.0 ± 0.11a	**0.79 ± 0.04b**	4.1 ± 0.18a

Table 4.2 Greenhouse. Variables by specific greenhouse trial, spring or fall, for Fuller and Duster. Treatment means within the same group (cultivar) with the same letters are not significantly different (*P* ≤ 0.05).

	Fuller		Duster	
	Spring- initiated	**Fall-initiated**	**Spring-initiated**	**Fall - initiated**
Culm Height (cm)	57.9 ± 1.2**a**	50.8 ± 1.1**b**	65.3 ± 0.6**a**	49.0 ± 1.0**b**
Spikelets/Spike	11.6 ± 0.3**a**	9.0 ± 0.2**b**	14.9 ± 0.2**a**	11.8 ± 0.3**b**
Seed Weight/Spike (g)	0.59 ± 0.02**a**	0.19 ± 0.02**b**	1.12 ± 0.03**a**	0.41 ± 0.05**b**
Seeds/Spike	1.6 ± 0.1**a**	0.9 ± 0.1**b**	2.1 ± 0.1a	2.2 ± 0.1a
Culms/Plant	5.7 ± 0.3a	5.0 ± 0.3a	3.0 ± 0.1**b**	5.9 ± 0.2**a**

Chapter 5 - Results Field Trial

Field Conditions

Unlike in the greenhouse, where data were analyzed by averaging collected data across the entire plant, results were analyzed on a culm bases in the field. This is because it was difficult to clearly determine what constituted an individual plant when harvesting the wheat. Field trials were conducted in two locations, one in Sedgwick County and one in Saline County. It is expected that there are different growing conditions (rainfall, temperatures, soil types, etc.) between locations that are over 120 km apart, and even in the same location environmental factors can vary greatly from one growing season to the next. However, these are not variables that are directly related to Hessian fly (HF) larval feeding and therefore these factors were blocked and analyzed. The results can be seen in Appendix A.

In the field setting it was not possible to conduct egg counts without damaging plants. Each year, one week after plots were infested, observations were made to determine if eggs were present on 10 random leaves. In all cases eggs were found, confirming that infestations did take place. However, due to harsh environmental conditions following infestations, including high winds and hot, dry weather that could desiccate small larvae as they moved from leaves to the plant sheath to begin feeding, heavy rains that could wash larvae, and possibly eggs off plants, it was impossible to determine the survival correlation between eggs to larvae. For this reason a culm was not considered infested unless at least one flaxseed was present at the time of data collection. The disadvantage for Armour and Duster is this shows only how plants that do not successfully kill HF larvae are impacted and is not able to determine if plants that may have been attacked by first instar larvae, but the larvae died due to the plants' resistance mechanisms, may have been impacted.

In either location, the percentage of infested culms never reached above 27.1%, even in the susceptible cultivar, Fuller (Table 5.1). In 2011, there were no HF flaxseeds found in any of the Fuller culms in Sedgwick County, while in Saline County, infestations in Fuller reached only 3.4% and in Armour were only 2.6%. The wheat growing season was particularly hot and dry that year. In 2011, Saline County was 11.46 inches under the annual precipitation average and received only 7.91 inches of rainfall during the active wheat growing season (NOAA 2014). In Sedgwick County the total annual precipitation was 6.58 inches below average with only 8.64 inches of rainfall during the active wheat growing season (NOAA 2014). Female flies that emerge in temperatures at or above 30°C will not produce viable eggs (Ratcliffe 2000). In Sedgwick County, plots were infested on 18 April 2011 when daytime temperatures reached 26°C. Plots in Saline Co. were infested on 28 April 2011 and while daytime temperatures reached only 23.9°C, the following day was 27.2°C. Although these temperatures may be below the 30°C threshold, they were considerably above the 21.1°C considered ideal for HF development and it was very dry and windy, thus may have decreased egg viability. This warm/hot, dry weather also likely desiccated eggs and/or larvae before they could establish themselves in sheltered feeding sites.

For the cultivar Duster in Sedgwick County, less than 1% of the culms were infested in any year and in Saline county only 3.8% of culms were infested (Table 5.1).

This small percentage of infested culms does appear to indicate that Duster is still resistant to HF larval feeding in the spring, post-vernalization. Interestingly, Armour had nearly the same percentage of infested culms as the susceptible cultivar, Fuller, and in 2010, actually had a higher percentage of infested culms (Table 5.1). This suggests that under field conditions, Armour may not provide any more resistance to HF larval feeding in the spring, post-vernalization than Fuller, a susceptible cultivar.

Culm height, number of spikelets and seeds per culm, and seed weight per culm

Under field conditions, Fuller, a cultivar considered to be highly susceptible to HF, were nearly 9 cm shorter when infested with HF larvae than non-infested plants (Fig. 5.1). In the intermediate and highly resistant cultivars Armour and Duster, respectively, those plants which contained at least one flaxseed at harvest were also found to have significantly shorter culms.

Culms from infested Fuller plants did not have more spikelets/spike than non-infested plants (Fig. 5.2). Infested culms in the cultivar Armour did have significantly fewer spikelets/spike, while the inverse was true for Duster where culms that were infested had significantly more spikelets/spike. Fuller and Armour had significantly fewer seeds/spikelet when culms contained at least one flaxseed (Fig. 5.3). In both Fuller and Armour, infested culms had significantly reduced seed weights (Fig. 5.4). There was no significant difference in the number of seeds/spikelet or seed weight/spike between infested and non-infested plants in Duster (Figs. 5.3 and 5.4). These results further support that HF larval feeding negatively impacts a susceptible cultivar Fuller, even under infestation levels of one adult female fly/tiller. The number of culms in the highly resistant cultivar Duster that contained a HF flaxseed was very low (1.3% on average) thus suggesting that this cultivar is still highly resistant to HF larval feeding under field conditions even after winter vernalization. On the other hand, Armour, a cultivar having intermediate, and often inconsistent resistance, did not appear to provide significant resistance to HF larval feeding under field conditions after winter vernalization. This was supported by the fact that, in field trials, Armour often contained a similar, and in one case a higher percentage of plants containing at least one flaxseed than the susceptible cultivar, Fuller (Table 5.1).

Figures and Tables

Figure 5.1 Field - Sedgwick and Saline Counties, averaged across all years. Average culm height (cm). Treatment means within the same group (cultivar) with the same letters are not significantly different (*P* ≤ 0.05).

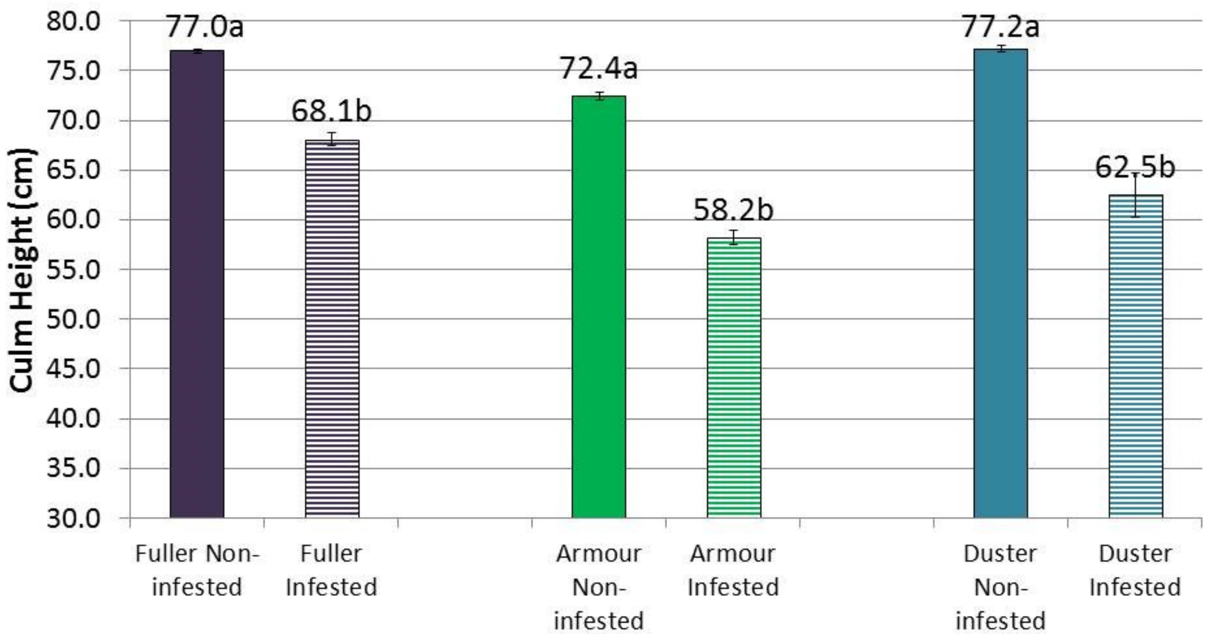

Figure 5.2 Field - Sedgwick and Saline Counties, averaged across all years. Average number of spikelets per spike. Treatment means within the same group (cultivar) with the same letters are not significantly different (*P* ≤ 0.05*)*.

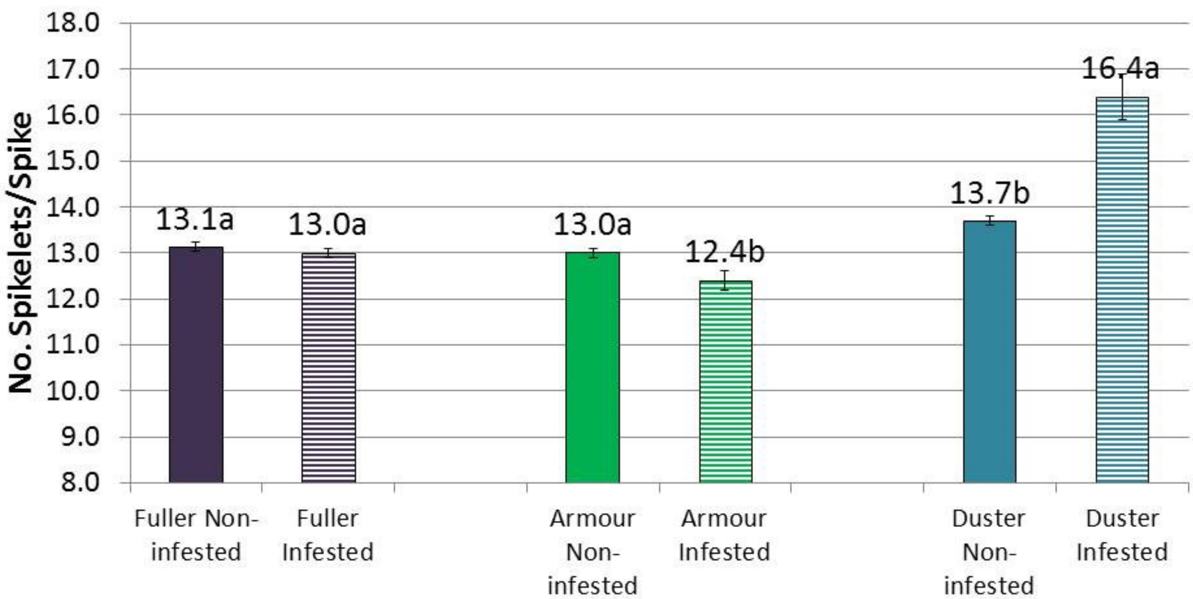

Figure 5.3 Field - Sedgwick and Saline Counties, averaged across all years. Average number of seeds/spikelet. Treatment means within the same group (cultivar) with the same letters are not significantly different (*P* ≤ 0.05*)*.

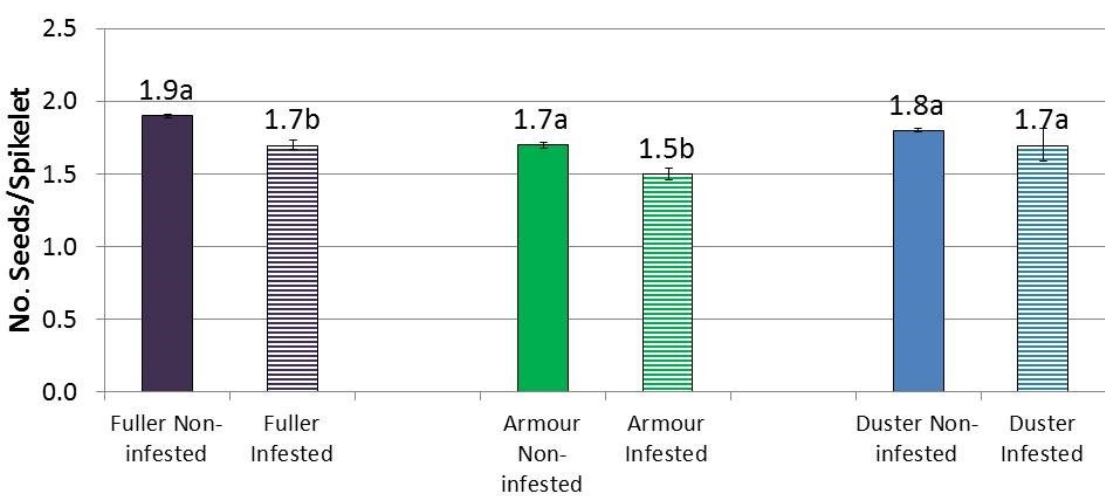

Figure 5.4 Field - Sedgwick and Saline Counties, averaged across all years. Average weight of seeds/spike. Treatment means within the same group (cultivar) with the same letters are not significantly different (*P* ≤ 0.05).

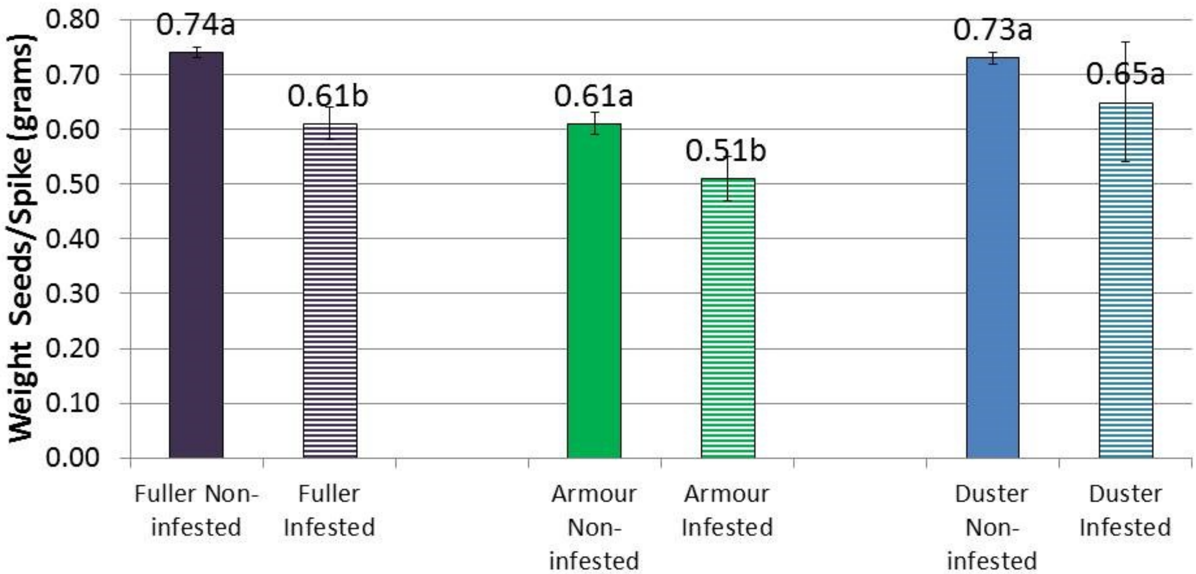

Table 5.1 Percent of culms infested with Hessian fly flaxseeds in Sedgwick and Saline Counties 2010 – 2012.

Sedgwick Co.			
	Fuller	**Duster**	
2010	5.3%	0.5%	
2011	0.0%	0.7%	
2012	27.1%	0.2%	
Saline Co.			
	Fuller	**Armour**	**Duster**
2010	17.1%	26.5%	
2011	3.4%	2.6%	
2012	15.9%	15.9%	
2013	21.0%	16.3%	3.8%

Chapter 6 - Discussion

The Hessian fly (HF) was a well-documented pest in Kansas in the early to mid-1900's (Horton et al.1945). Use of the Hessian Fly-Free Date, resistant wheat cultivars, and other cultural control methods such as destroying volunteer wheat, tillage, etc. helped reduce this pest to acceptable levels for Kansas growers. However, in the early 1990's HF once again began to increase to levels capable of causing significant damage to wheat fields. A preliminary study hypothesized that possibly fall temperatures in Kansas had increased to a level that allowed the HF populations to thrive longer into the year than previously possible and possibly allowing for an extra generation each year. Thermal unit requirements for the HF have been well studied and it is known that 343 thermal units above 1.6°C are needed for the complete development of the larval and flaxseed stages (Foster and Taylor 1975). However, average fall temperatures from 1900 to 2008 in Riley, Smith, Phillips, and Sedgwick Counties fluctuated greatly from year to year, but did not show any trend for an overall increase or decrease, which would help explain the more numerous HF infestations because of the additional thermal units to allow significantly more HF development (Davis et al. 2009). This also indicates that temperature is not likely playing an important role in the recent increase in HF populations and begs the question as to what factor(s) may be playing a role and how this increased population may be impacting Kansas growers.

Once HF sex pheromone became commercially available it was feasible to re-examine the historically established HF-free date and monitor HF populations throughout the year to better understand when flies were active. Thus, the opportunity was provided to re-examine the previously reported idea that two main broods occurred in Kansas, one in fall and one in summer. However, under this intense sampling it was instead determined that flies are constantly emerging and that there may be multiple small population peaks throughout the spring, summer, and fall (Dean and McCullough 1915). Based on the results of this study, it appears that HF populations in Kansas may be more similar to those found in the South where there may be two to three generations during the spring and fall with emergence of some individuals from each generation being delayed so that there may be small numbers of flies emerging almost continually (Morgan et al. 2005). However, we were not able to support their claim that adult flies will emerge from pupae approximately 12 days after a moisture event. When rearing HF in the lab, after the flaxseeds are removed from cold storage, the material is kept moist in order to maximize adult emergence (Dendy 2009). While it is known that allowing the flaxseeds to completely desiccate will lead to death, the required moisture and relative humidity for survival of the flaxseed and emergence of the adult in the field is not known. Pheromone trapping is currently being conducted in Reno and Dickinson counties in KS to more specifically delineate, hopefully, how moisture events impact adult HF emergence.

In all Kansas counties where fall HF pheromone trapping was conducted, flies were captured at least one month after the historical HF-free date, thus indicating the historically used fly-free date is no longer accurate and should be referred to as the 'Best Pest Management Date'. We do not recommend the management tool be discarded altogether as, in general, later planting may help to reduce the impact of many wheat pests including aphids, wheat curl mites, and many diseases (Michaud et al. 2014). Additionally, we were not able to determine if these male flies moving around this late in the fall were actively mating and if those females were laying viable eggs. And, if the males were mating and females producing viable eggs were they able to survive overwinter? While extensive work has not been done to determine this, a HF flaxseed was collected on 1 Dec, 2009 in Sedgwick County, KS that was unsclerotized (Jeff Whitworth, personal communication). Within 24 hours this flaxseed hardened and turned the characteristic reddish color and presumably would have successfully emerged as an adult, suggesting at least some of these late-flying HF are producing viable offspring.

Results of spring HF pheromone trapping also provided surprising results. In Kansas, growers most commonly consider the HF a fall problem and threat to seedling wheat. The development of resistant wheat cultivars has provided good protection for seedling plants in the fall. However, our results show that HF is active throughout the spring, often in rather large numbers, and therefore may be a real threat to wheat in the spring after winter vernalization. At the time this study was initiated, there was no known research documenting if hard red winter wheat cultivars, screened for resistance at the seedling stage, were still providing protection to plants when they were more mature, post-winter vernalization.

In this study, the intermediately resistant cultivar, Armour, produced some interesting differences in results whether from the greenhouse or from the field. In greenhouse trials it appeared that Armour was resistant to HF larval feeding with no significant reductions seen for any of the variables except seed weight, and this was found only under the infestation level of 3 flies/tiller. Loss of seed weight, however, equals loss of yield and therefore is likely the most important variable to growers. Additionally, very few plants in the greenhouse were found to be infested with flaxseeds, demonstrating that few larvae were able to successfully feed and survive to the pupal stage on Armour. In contrast, Armour grown and infested in the field in spring appeared to lose resistance to HF larval feeding as there were significant losses in culm height, the number of spikelets/spike and the number and weight of seeds. The percentage of plants found to be infested with HF flaxseeds in the field was similar to that found in the susceptible cultivar, Fuller, showing that HF larvae were able to successfully feed and develop on these plants. While HF resistance may have held up in the greenhouse under ideal temperature, moisture, light, and nutrient conditions, the temperature extremes or other stressors found in the field may have caused Armour to lose its resistance. In many plant-pathogen relationships, heat stress is known to reduce plant immunity (Zhu et al. 2010). Currie et al. (2014) demonstrated that a significant number of seedling plants of the cultivar, Molly lost resistance to HF populations after a transient heat stress event. Although it has been shown that seedling plants may regain their resistance after a heat stress event, it is unknown how plant resistance will hold up past the seedling stage and when heat stress events may be frequent or long-lasting, nor is it known how extreme cold may impact plant resistance (Liu et al. 2013). The reason Armour plants maintained their HF resistance in the greenhouse but lost it under field conditions may be related to the frequent hot temperatures plants were exposed to in the field during and around the time plants were under attack by HF larvae. Therefore, under conditions where growers know they have chronic HF populations, Armour is not likely to be a cultivar recommended for planting to protect against HF.

In the greenhouse trials, it was found that Duster, a cultivar with HF resistance, showed a significant loss in culm height and number of spikelets/spike when infested with 1 fly/tiller but that these losses were not found under heavier infestations of 3 flies/tiller. Similar results were found in the field where the only variable significantly impacted was the height of culms, which was significantly reduced in infested plants. In the greenhouse the weight of seeds actually increased significantly when plants were infested with 3 flies/tiller. Increased plant height, number of heads produced, number of seeds produced, and seed weight in resistant wheat cultivars, under HF pressure has been documented in a previous study (Anderson et al. 2011), and while the exact cause of this is not known, it may be related to genetic and metabolic changes that Duster makes as a reaction to HF larval feeding. In addition, the low number of HF flaxseeds found in Duster plants both in the greenhouse and in the field, support that Duster is highly resistant to HF larval feeding, even in the spring, post-vernalization. Results of this study indicate there is a need for further study of all commercially available wheat cultivars with varying levels of HF resistance to determine which cultivars may still provide good protection to wheat in the spring, post-vernalization.

Even though HF resistant cultivars are commercially available, and are being planted in Kansas, the majority of wheat acreage planted each year is still HF susceptible, or provides only intermediate resistance (Lingenfelser 2013), which we have indicated may not hold up in the spring. Therefore, it is important to determine how HF larvae, feeding in the spring, may impact wheat. Typically, growers are not aware that HF are present in their fields until, close to harvest, wheat lodges, creating a drastic example of the damage this pest can do. However, severe outbreaks leading to significant lodging are not common in Kansas every year. But, small populations of flies are present in most, if not all, wheat fields in north central, central, and south central Kansas every year (Jeff Whitworth, personal communication). Yet, we do not understand how these small, but consistent populations of flies may be impacting wheat. In this study, in the HF susceptible cultivar, Fuller, it was found in both the greenhouse and field trials that larval feeding had a negative impact on plant height, number of spikelets, and the number and weight of seeds. These data support the findings of a study by Ratcliffe and Hatchett (1997) where they reported that wheat attacked by HF during stem elongation produced heads with lower seed weights and fewer seeds. In the greenhouse portion of this study, the number of culms/plant actually increased under HF infestations. Previous studies have shown that wheat infested with HF during the seedling stage often produces more tillers than non-infested wheat (Byers and Gallun 1971, Anderson et al. 2011). Byers and Gallun (1971) found that the length of time larvae were allowed to feed on seeding plants (2 or 3 days) had a larger impact on leaf elongation and seedling plant weight than the number of larvae feeding, whether 1-5 or 5-10 larvae/plant. Information provided by this study confirms the feeding of just one HF larva can cause irreparable damage to a plant, not only in the seedling stage, but also in more mature plants, post-vernalization.

For the end user, growers in Kansas, the significant loss of seed weight due to HF larval feeding is probably the most important factor. While most growers are aware that significant HF infestations can lead to seedling death in the fall and/or plant lodging in the spring, yield loss due to spring populations of HF that do not lead to plant lodging are unknown. Past studies of soft red winter wheat grown in the southern U.S. have reported yield losses of 6.05 kg/ha to 11.8 kg/ha for each 1% of infested stems in the spring (Hill and Smith 1925, Buntin 1999). In this study, we found an average yield loss of 0.13g/spike or 65kg/ha (1 bu/acre) in the susceptible cultivar, Fuller when comparing infested versus non-infested plants in the field. In the greenhouse, plants infested with just 1-5 flaxseeds lost an average of 0.09g/spike or 44kg/ha (0.7bu/acre) compared to non-infested plants. This means that a grower could be losing significant yield without ever being aware that HFs are active in the field.

Spring and summer pheromone trap catches from this study also provide support for the importance of controlling volunteer wheat. Emerging HF adults from wheat stubble may simply move into volunteer wheat to mate and lay eggs. Under favorable conditions this may lead to an additional generation and will increase HF populations, which may then become an increasingly significant problem in winter wheat. These emerging flies may also be able to move into other native and introduced grasses to deposit eggs, continue development, and increase populations that may infest winter wheat when it is planted in the fall (Zeiss et al. 1993, Chen et al 2009b).

In the greenhouse trials, the interactions between selected plant growth stages infested and the HF infestation level did not follow a clear pattern. In the cultivar Armour, no significant differences were found. In Fuller however, it was found that plants infested earlier in their development (Feekes 7-8) with 3 flies/tiller had significantly fewer spikelets/spike, fewer seeds with lower seed weights, and fewer culms/plant than plants infested at either growth stage with 1 fly/tiller or non-infested. Conversely, both the non-infested plants and those infested with 1 fly /tiller had significantly more spikelets/spike in the later planted/ infested (Feekes 7-8) than in the earlier planted infested (Feekes 9-10). The fact that the non-infested plants and those infested at the lower rate both had more spikelets at the earlier planting and/or infestation date may suggest this is related more to plant physiology than HF larval feeding, while the significant reduction in spikelets/spike when plants were infested earlier (Feekes 7-8) under increased HF feeding may indicate the timing of HF infestations during the plant growth may play a role in how severely the insect feeding impacts the plant. These results indicate that under greater HF infestations, the earlier in development wheat is attacked the more severe the losses may be. In the cultivar Duster, it was found that for both the non-infested plants and those infested with 1 fly/tiller, plants planted earlier or infested at Feekes 9-10 had significantly more spikelets/spike than those planted later or infested at Feekes 7-8, although no difference was found for plants infested with 3 flies/tiller. As this phenomenon was found in un-infested plants it suggests that any significant differences are the result of an unrelated and undetermined factor rather than as a result of HF feeding. Similar inconsistencies were recorded with seed weight and number of culms/plant.

In conclusion, the results of this study demonstrate the historically established Hessian Fly-Free Date is no longer valid in Kansas to avoid HF infestation. The HF-Free Date should therefore be referred to as the Best Pest Management Date as it may still help mitigate other pest and disease problems. HF males are also active, in larger numbers and much later into the spring than previously thought and, therefore HF larval feeding may be a serious threat to Kansas wheat in the spring, post-winter vernalization. It was found that Duster, a cultivar rated as highly resistant, appears to still provide good protection to plants in both the greenhouse and field. Armour, a cultivar with intermediate resistance, may provide good protection to plants in a greenhouse setting under small infestation levels. However, under larger infestations or in a field setting plants begin to lose yield. Additionally, the percentage of Armour plants infested with at least 1 flaxseed/tiller at harvest, in the field, was not different from plants of the susceptible cultivar, Fuller. In addition, those infested plants were significantly shorter with fewer spikelets, a lower number of seeds, and reduced seed weights. This indicates that all cultivars being released as HF resistant should be screened post-vernalization to determine if they are still providing good plant protection. This study also found that Fuller, a HF susceptible cultivar, attacked by HF in the spring during the flag leaf stage of development, may result in significant yield losses that growers are currently not aware of and should be taken into consideration. Pheromone trapping could be a good method for growers to monitor wheat fields to determine if HF are a potential source of yield loss that may need to be managed. Additional research to delineate how pheromone trap catches correlate to infestation levels would add significantly to a better understanding of the Kansas wheat/Hessian fly relationship.

Chapter 7 - References

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Appendix A - Hessian fly field trials

Table A.1 Field. Results of other varieties infested in the field. Treatment means within the same group (cultivar) with the same letters are not significantly different (*P* ≤ 0.05).

Wheat Cultivar and Hessian Fly infestation level	Culm Height (cm)	Spikelets/Spike	Seeds/Spikelet	Seed Weight/Spike (grams)
Post Rock - Non-infested	73.9 ± 0.3a	14.2 ± 0.1a	1.7 ± 0.02a	0.71 ± 0.01a
Post Rock - Infested	62.2 ± 3.1b	15.3 ± 0.9a	1.0 ± 0.2b	0.27 ± 0.14b
TAM 204 - Non-infested	51.1 ± 0.8a	11.5 ± 0.2a	2.3 ± 0.05a	0.76 ± 0.03a
TAM 204- Infested	34.3 ± 4.8b	9.4 ± 1.3a	1.3 ± 0.3b	0.26 ± 0.22b
Jackpot - Non-infested	47.5 ± 0.9a	11.1 ± 0.2a	1.7 ± 0.04a	0.59 ± 0.03a
Jackpot - Infested	31.8 ± 5.0b	9.8 ± 1.2a	1.7 ± 0.2a	0.25 ± 0.17a

Table A.2 Differences found between years (locations combined) and locations (years combined). Treatment means within the same group (culm height, no. spikelets, no. seeds, seed weight) with the same letters are not significantly different ($P \leq 0.05$).

	Culm height (cm)	No. Spikelets/ Culm	No. Seeds/ Spikelet	Seed Weight/Spike (grams)
2010	65.0 ± 0.3d	12.5 ± .07c	2.0 ± 0.02a	0.76 ± 0.02b
2011	71.4 ± 0.3c	13.5 ± .07b	1.8 ± 0.02b	0.84 ± 0.02a
2012	82.0 ± 0.3a	12.6 ± .06c	1.8 ± 0.01b	0.58 ± 0.01c
2013	78.5 ± 0.3b	14.4 ± .07a	1.6 ± 0.02c	0.54 ± 0.02c
Saline County	78.7 ± 0.2a	13.0 ± .06b	1.6 ± 0.02b	0.55 ± 0.01b
Sedgwick County	69.9 ± 0.3b	13.5 ± .05a	2.0 ± 0.01a	0.81 ± 0.01a

Appendix B - Hessian fly pheromone trapping

Figure B.1 Pheromone trap data for Dickinson County 8 October to 7 December, 2010. Each trap catch represents the total of 2 traps. Blue line represents 24 hour precipitation totals (cm), green line represents the high relative humidity recorded during that 24 hour period (%).

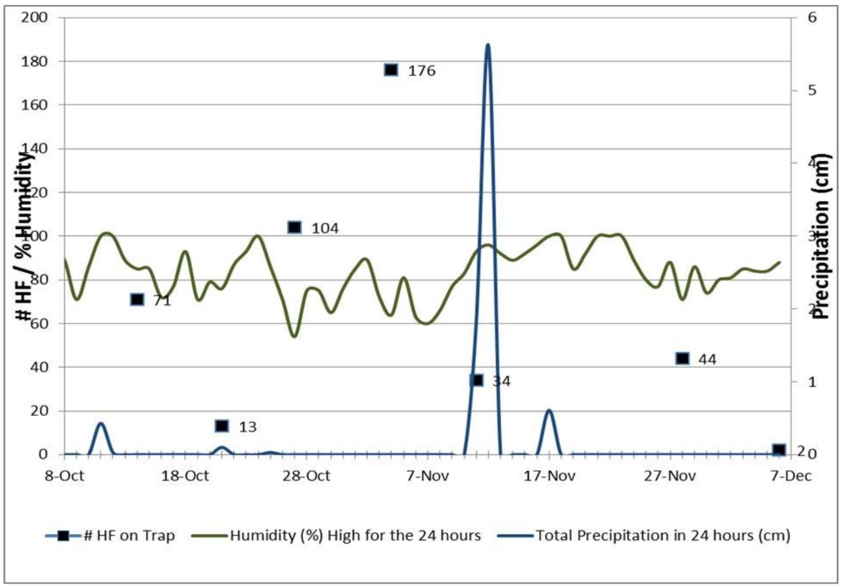

Figure B.2 Pheromone trap data for Dickinson County 1 April to 3 July, 2011. Each trap catch represents the total of 2 traps. Blue line represents 24 hour precipitation totals (cm), green line represents the high relative humidity recorded during that 24 hour period (%).

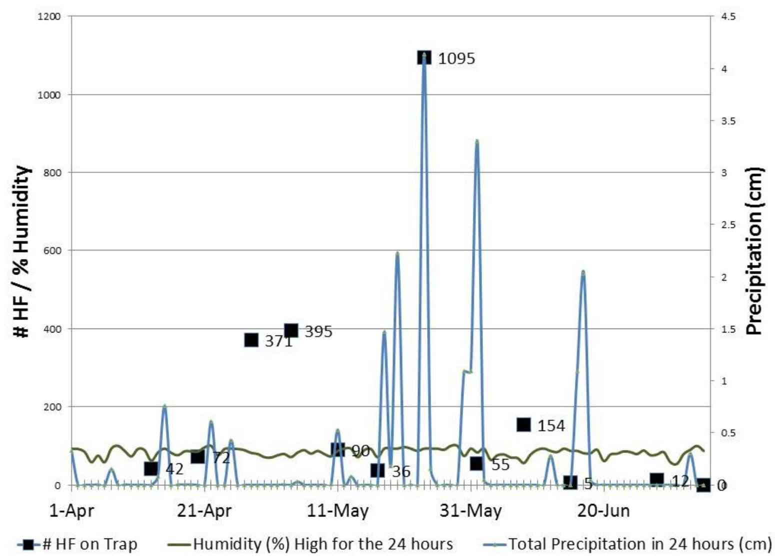

Figure B.3 Pheromone trap data for Saline County 7 November to 2 December, 2009. Blue line represents 24 hour precipitation totals (cm), green line represents the high relative humidity recorded during that 24 hour period (%).

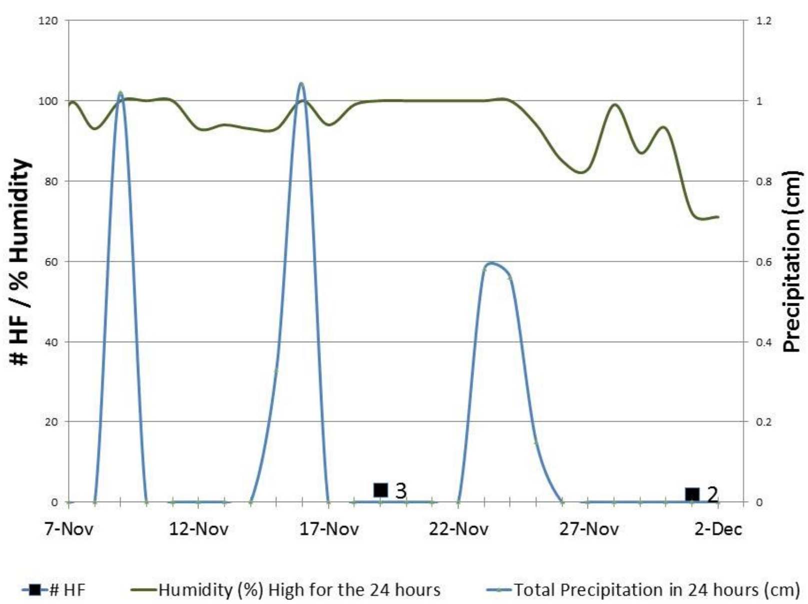

Figure B.4 Pheromone trap data for Saline County 16 April to 20 June, 2010. Blue line represents 24 hour precipitation totals (cm), green line represents the high relative humidity recorded during that 24 hour period (%).

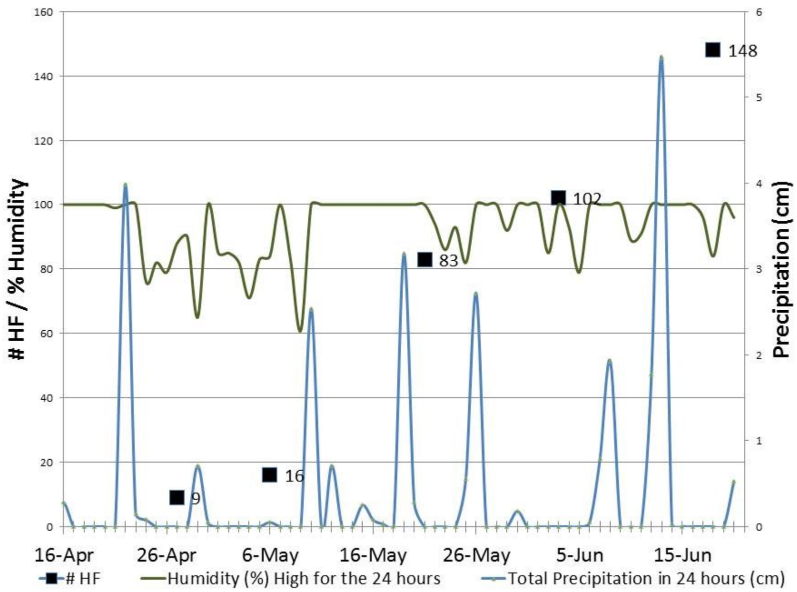

Figure B.5 Pheromone trap data for Sedgwick County 25 September to 29 November, 2006. Trap data collected by Aqeel Ahmed and Gary Cramer. Blue line represents 24 hour precipitation totals (cm), green line represents the high relative humidity recorded during that 24 hour period (%).

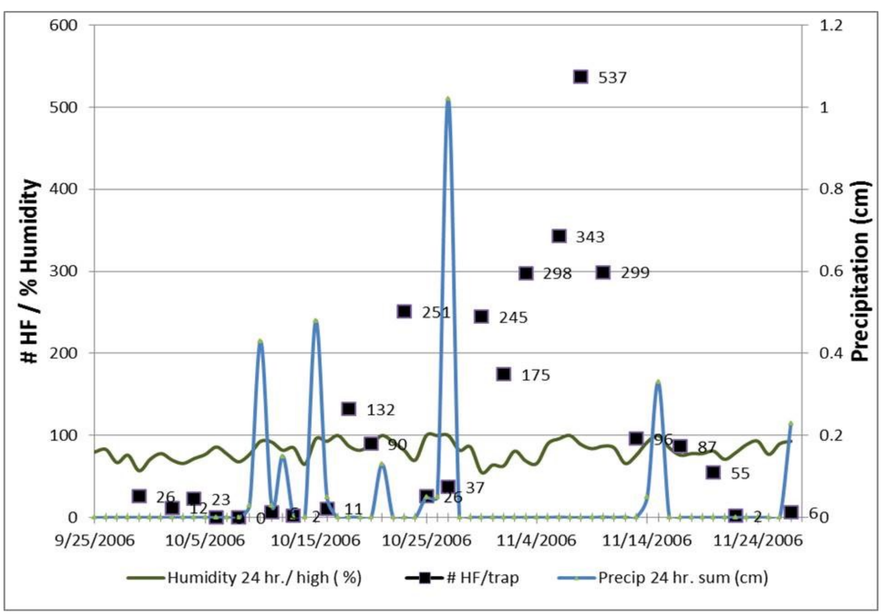

Figure B.6 Pheromone trap data for Sedgwick County 30 September to 14 November, 2007. Trap data collected by Aqeel Ahmed and Gary Cramer. Blue line represents 24 hour precipitation totals (cm), green line represents the high relative humidity recorded during that 24 hour period (%).

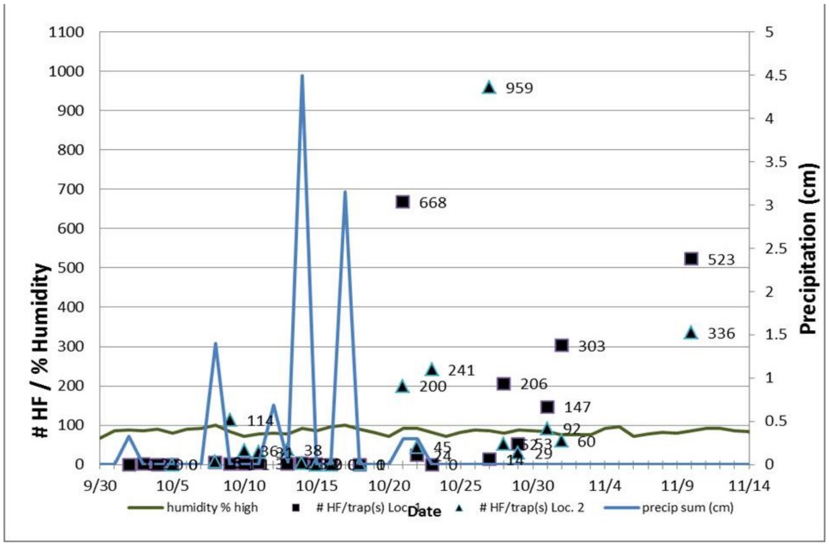

Figure B.7 Pheromone trap data for Sedgwick County 11 April to 10 June, 2009. Blue line represents 24 hour precipitation totals (cm), green line represents the high relative humidity recorded during that 24 hour period (%).

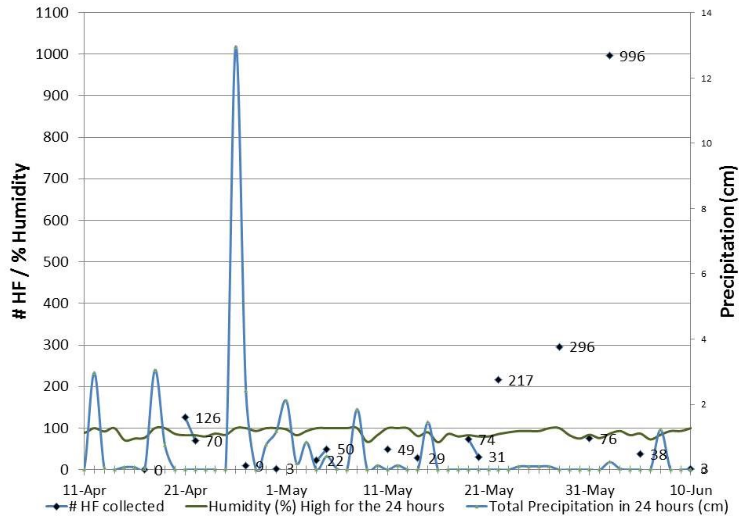

Figure B.8 Pheromone trap data for Sedgwick County 27 October to 5 December, 2009. Blue line represents 24 hour precipitation totals (cm), green line represents the high relative humidity recorded during that 24 hour period (%).

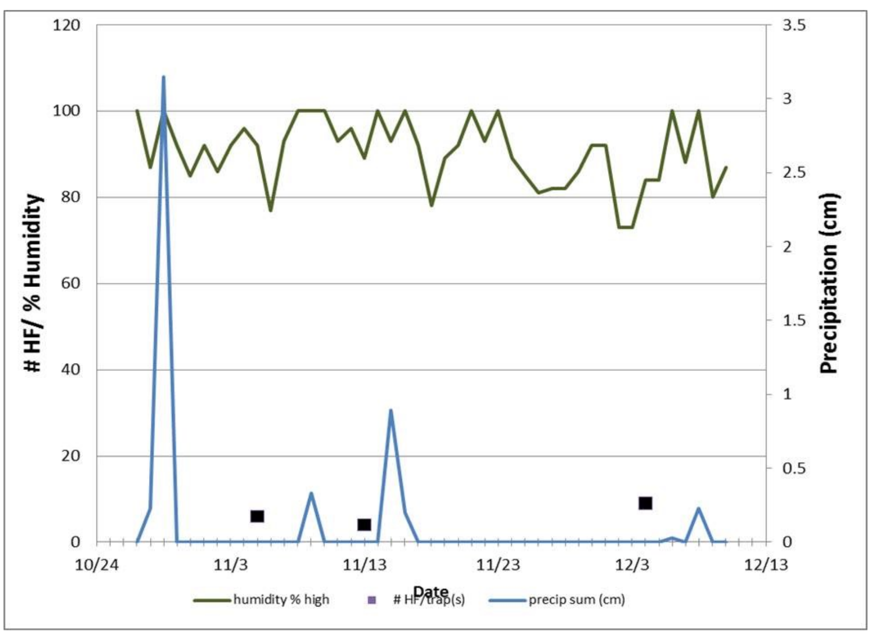

Figure B.9 Pheromone trap data for Sedgwick County 6 May to 26 May, 2010. Blue line represents 24 hour precipitation totals (cm), green line represents the high relative humidity recorded during that 24 hour period (%).

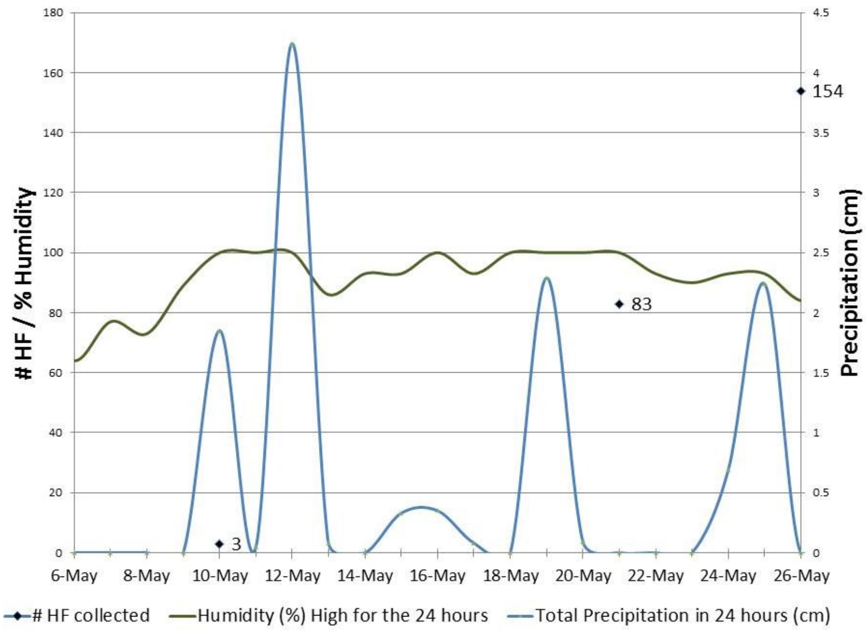

Figure B.10 Pheromone trap data for Sedgwick County 9 September to 8 December, 2010. Blue line represents 24 hour precipitation totals (cm), green line represents the high relative humidity recorded during that 24 hour period (%).