

Hessian fly, *Mayetiola destructor* (Diptera: Cecidomyiidae), smart-trap design
and deployment strategies

by

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B.S., South Dakota State University, 2011
M.S., South Dakota State University, 2014

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Abstract

Timely enactment of insect pest management and incursion mitigation protocols requires development of time-sensitive monitoring approaches. Numerous passive monitoring methods exist (e.g., insect traps), which offer an efficient solution to monitoring for pests across large geographic regions. However, given the number of different monitoring tools, from specific (e.g., pheromone lures) to general (e.g., sticky cards), there is a need to develop protocols for deploying methods to effectively and efficiently monitor for a multitude of potential pests. The non-random movement of the Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), toward several visual, chemical, and tactile cues, makes it a suitable study organism to examine new sensor technologies and deployment strategies that can be tailored for monitoring specific pests. Therefore, the objective was to understand Hessian fly behavior toward new sensor technologies (i.e., light emitting diodes (LEDs) and laser displays) to develop monitoring and deployment strategies. A series of laboratory experiments and trials were conducted to understand how the Hessian fly reacts to the technologies and how environmental factors may affect the insect's response. Hessian fly pupae distribution within commercial wheat fields was also analyzed to determine deployment of monitoring strategies. Laboratory experiments demonstrated Hessian fly attraction to green spectrum (502 and 525 nm) light (LEDs), that response increased with light intensity (16 W/m^2), and that they responded in the presence of wheat odor and the Hessian fly female sex-pheromone, but, response was reduced under ambient light. These laboratory experiments can be used to build a more targeted approach for Hessian fly monitoring by utilizing the appropriate light wavelength and intensity with pheromone and wheat odor to attract both sexes, and mitigating exposure to ambient light. Together this information suggested that light could be used with natural cues to increase attraction. Therefore,

a light source (green laser display) was applied to a wheat microcosm, which resulted in greater oviposition in wheat covered by the laser display. Examination of Hessian fly pupal distribution within commercial wheat fields showed that proportion of wheat within a 1 km buffer of the field affected distribution between fields. This helps to inform deployment of monitoring strategies as it identified fields with a lower proportion of wheat within a 1 km buffer to be at higher risk Hessian fly infestation, and therefore monitoring efforts should be focused on those fields. Together this work demonstrates Hessian fly behavior toward new sensor technologies, how those technologies interact with environmental cues, and how environmental composition affects pupal distribution. Collectively this information will enable cheaper, more accurate and more efficient monitoring of this destructive pest.

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Chapter 1 - Literature Review

Introduction

The Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), is present in most of the wheat (*Triticum* spp. L. (Poales: Poaceae)) producing regions of the world (CABI 2016). This fly is one of the oldest documented invasive species to North America, first reported in New York in 1779 (Pauly 2002). While adults do move throughout the landscape, their short adult lifespan (1 – 4 days) limits their ability to disperse across geographic barriers (e.g., ocean) (Bergh et al. 1990, Harris and Rose 1991, Withers et al. 1997). Therefore, movement of wheat straw infested with Hessian fly pupae is a likely source of incursion to new regions, as is reported to have occurred for the introduction of Hessian fly into North America (Pauly 2002). Multiple introductions of the Hessian fly to the U.S. have likely occurred (Morton and Schemerhorn 2013), resulting in the presence of Hessian fly in most wheat (a common host) growing regions of the U.S. (Ratcliffe and Hatchett 1997, Ratcliffe et al. 2000). The Hessian fly is a member of one of the largest family of flies, Cecidomyiidae (Gagne 1994), which contains many economically important species, including the sorghum midge, *Contarinia sorghicola* (Coquillett) (Diptera: Cecidomyiidae), and the sunflower midge, *C. schulizi* Gagne (Diptera: Cecidomyiidae) (Harris et al. 2003, Stuart et al. 2008). Gall formation is a feature associated with flies in this insect family (Harris et al. 2003), which provides a protective structure and induces a nutritive rich feeding site for larvae (Rohfritsch 1992). This has enabled the Hessian fly to become a major pest of wheat in the U.S. (Harris et al. 2003, Whitworth et al. 2009, Flanders et al. 2013). This article summarizes common management practices used to combat this pest, along with life history, host range, and dispersal behavior important to the

understanding of Hessian fly management. The tactics reviewed can be used as part of a Hessian fly IPM program.

Geographic Distribution and Host Range

The Hessian fly originated in the Fertile Crescent region of the Middle East and is now present in Europe, North Africa, North America, and New Zealand (Stuart et al. 2012). Many grass species serve as hosts of this fly (Zeiss et al. 1993a), including at least 16 wild grass species found around the world (Harris et al. 2001), most belonging to the tribe Triticeae. Triticeae includes major cereal crops such as wheat, barley, and rye, but wheat is the optimum host for population increase (Harris et al. 2001, Chen et al. 2009a). In choice tests, Hessian fly prefers wheat, followed by rye, then barley (Harris et al. 2001, Chen et al. 2009a). Grasses in the tribe Bromeae are also hosts for Hessian fly in New Zealand (Prestidge 1992, Stewart 1992).

Life Stages

Adult. Hessian fly adults are brown or black, with females at times appearing reddish-brown owing to the presence of the orange eggs developing inside the abdomen (Fig. 1.1) (VanDuyn et al. 2003, Foster and Hein 2009). There are taxonomic keys to distinguish Hessian fly from related species that appear similar; e.g., Manual of Nearctic Diptera (McAlpine et al. 1981, Toolbox 2010).

Egg. Eggs (Fig. 1.2A) are found in the grooves on the upper side of the plant leaf, and take 3 – 12 days to hatch depending on the temperature, 50° – 85°F (10° – 29°C) (McColloch 1923, Packard 1928). Hessian fly eggs can be recognized by their orange color, elliptical shape, and small size (Flanders et al. 2013).

Larvae. Larvae are white, cylindrical, and develop a translucent green stripe down the middle of the back (Fig. 1.2B) (Gagne and Hatchett 1989, Flanders et al. 2013). There are three

larval instars, and larval size varies between instar stages, doubling in length from the first (0.56 – 1.70 mm) to the second instar (1.70 – 4.00 mm) (Gagne and Hatchett 1989).

Pupa/Puparia. The third-instar and pupae develop in the cuticle of the second-instar larva (Gagne and Hatchett 1989), termed the puparium. This stage is commonly referred to as the flax seed stage (Fig. 1.2C), due to the hardened, sclerotized, dark brown color, and shape of the cuticle, which resembles a seed of flax, *Linum usitatissimum* L. (Malpighiales: Linaceae).

Biology and Life History

Development and Establishment on Host. The Hessian fly can complete its life cycle in as few as 28 days, but development can be delayed during long periods of aestivation and diapause based on temperature. Upon hatching in seedling wheat, first instar larvae move towards the base of the plant using parallel venation in the leaf as a guide and establish a feeding site on the stems within the plant crown (Stuart et al. 2012). In wheat plants that have elongated stems, the neonate larva establishes a feeding site on the stem beneath the leaf sheath at a node. Neonates require 12 – 24 h to move from the egg to the feeding site and larval mortality during this transit is high due to relative humidity, wind, cold, and rainfall (Packard 1928, Hamilton 1966). Only first and second instar larvae feed, which lasts for two to three weeks (Foster and Hein 2009). The length of the third-instar/pupal stage typically ranges from 7 – 35 days depending on environmental conditions; unless the pupae enter a long dormant period known as aestivation (over-summer) or diapause (over-winter) when temperatures are too warm or cold to trigger eclosure of adults, respectively (Foster and Taylor 1975, Wellso 1991, Morgan et al. 2005, Chapin 2008). Adults typically eclose (emerge) after 10 – 14 days if temperatures remain $\geq 21^{\circ}\text{C}$ (70°F), the optimum temperature for Hessian fly growth and development (Foster and Taylor 1975). High humidity and moisture in the surrounding environment must also be present

for adult eclosure, although these conditions have not been quantified (Morgan et al. 2005, Stuart et al. 2012). Once a Hessian fly adult ecloses from the puparia, it will live for 1 – 4 days (Bergh et al. 1990, Harris and Rose 1991). During that time, females mate and oviposit on suitable host plants.

Since Hessian fly development, aestivation, and diapause are dependent on temperature and moisture, the number of generations varies across the regions of the United States. In the Northern states (Kansas, Missouri, Pennsylvania, and Nebraska), Hessian fly completes at least two generations every year, one spring and one fall (Boyd and Bailey 2000, Foster and Hein 2009, Whitworth et al. 2009, Tooker 2012). In the Southern U.S. (from Texas to the Carolinas), Hessian fly completes three to six generations each year, 1 – 3 in the fall and 1 – 3 in the spring (Lidell and Schuster 1990, Morgan et al. 2005, Flanders et al. 2013). Identifying generations is difficult as all individuals of a generation do not emerge as adults during the same time. Rather, individuals continue to emerge as adults for up to a year; therefore, a cohort of Hessian fly is termed a brood as it may be composed of progeny from several previous generations (McColloch 1923, Wellso 1991). The number of generations or broods, especially during the fall, has important implications for Hessian fly management strategies, specifically for delayed planting. Northern states typically can utilize delayed planting as an effective management strategy (Whitworth et al. 2009, Tooker 2012, Knodel et al. 2018), while delayed planting is less effective in the Southern states (Buntin and Chapin 1990, Morgan et al. 2005, Bradford 2014). Delayed planting for Hessian fly management is discussed in greater detail in the Management Options section.

Dispersal and Flight. The Hessian fly has expanded its range to four continents and New Zealand (Stuart et al. 2012), making it highly successful at invading new regions of the world;

however their spread has been greatly aided by human movement and trade. On local landscape levels, wind and plant community composition are major factors affecting localized dispersal of Hessian fly. Early observations of Hessian fly infestations in Kansas fields concluded that adults, especially mated females, may be carried at least 3.2 km by the wind because no wheat or other hosts were within 3.2 km of the infested fields (McColloch 1917). More recently Withers et al. (1997) observed that ovipositing female Hessian flies disperse at a greater rate through areas of non-host plants than areas with host plants. Wind speed also affects dispersal of female Hessian flies. As wind speed increases, females exhibit more downwind flights (likely due to their inability to fly against stronger winds), while remaining on plants for longer periods of time (Withers and Harris 1997). Additionally, Harris and Foster (1991) found that male Hessian flies exhibit upwind flight when exposed to components of the female Hessian fly sex pheromone. While the Hessian fly has been documented to move between a few meters to a few kilometers (McColloch 1917, Withers et al. 1997), wind likely plays a major role in local dispersal between wheat fields. However, what is not clear is the average or maximum dispersal range of adult Hessian flies, as observations of Hessian fly movement beyond a few meters are based on assumptions of the source plants (McColloch 1917). Knowledge of average and maximum distance travelled by adults will be important to understanding Hessian fly movement within the local landscape, field to field dispersal, or movement within a field.

Studies to date suggest that Hessian flies exhibit non-random movement, demonstrated by directed flights toward a pheromone, plant extract, a certain wavelength of color, or spatial configuration (Harris and Rose 1990, Withers et al. 1997, Anderson et al. 2012). Flight behavior is adjusted when changes in plant distribution is sensed through the use of chemoreceptors for host-specific chemicals, where females are much more likely to stay in an area when wheat is

detected as opposed to a less attractive plant such as oats (Withers and Harris 1996). In other words, Hessian flies choose when and where to move based on cues from the environment.

Environmental Dispersal Cues. Females find their host plant using chemical, visual, and tactile cues (Harris and Rose 1990). Physical characteristics of hosts, such as the number and depth of vascular grooves, are also important for oviposition site selection (Kanno and Harris 2000a, b). The adaxial (upper) surface of the youngest leaf of the plant is the preferred oviposition site (Kanno and Harris 2000b, Ganehiarachchi et al. 2013). Larval survival is highest on the youngest leaf of the plant, and it is thought that the larvae need access to ‘reactive’ cells, which are plant cells easily manipulated to use as a food source (Ganehiarachchi et al. 2013).

Visual stimulants consisting of spectral and spatial information are important for attracting the fly from a distance. Females are attracted to the color green (530 – 560 nm) and brighter colors (Harris and Rose 1990, Harris et al. 1993). Besides spectral discrimination, females also approach and land more frequently on targets with vertical rather than horizontal contour lengths (i.e., horizontal lines) especially vertical lengths with higher density (Harris et al. 1993). The attraction of female Hessian flies to vertical contour lengths is not surprising, as a typical profile of wheat consists of many vertical contour lengths. While visual cues are important for Hessian fly oviposition, chemical cues are equally important.

Laboratory tests have shown Hessian flies preference for specific extracts of host plants, particularly wheat extract (Harris and Rose 1990, Kanno and Harris 2000a). Their attraction to plant extracts can be enhanced when combined with other physical attractants. For example, combinations of wheat extract with color and/or tactile attractants results in a greater number of eggs laid by the Hessian fly than any of the three attractants alone (Harris and Rose 1990).

The female sex pheromone is another strong olfactory cue that attracts male Hessian flies (Morris et al. 2000, Andersson et al. 2009). A synthetic female sex-pheromone was developed by Andersson et al. (2009). The pheromone lure has been tested in laboratory bioassays, small plot tests, and field tests and has been shown to be effective at attracting male Hessian flies (Anderson et al. 2012, Knutson et al. 2017). However the effective range of the pheromone is not known, partly because the average daily dispersal range of the Hessian fly has not been quantified.

Associated Injury and Damage

Injury to wheat caused by feeding manifests itself in the form of a darker, almost blue-green, foliage color (Fig. 1.3D) and stunted growth (Whitworth et al. 2009). Seedlings sometimes compensate by increased tillering, but continued feeding on the plant will decrease growth of additional tillers (Anderson and Harris 2006, Anderson et al. 2011, Stuart et al. 2012). Yield loss caused by larval feeding on seedlings (Fig. 1.2B) results from stunted growth and death of tillers and seedlings (Figs. 1.3A, C). Larval feeding on wheat after stem elongation, causes lodging from weakened stems, failure to produce a seed head, and a reduction in the number of seeds per spike and seed weight (Buntin 1999, Harris et al. 2003, Schwarting et al. 2016) (Figs. 1.3A, B, C).

In the U.S., the Hessian fly is a potential economic pest in many of the wheat production regions (Smiley et al. 2004, Watson 2005, Alvey 2009). Damage caused by Hessian fly feeding can result in significant yield loss. From 1984 – 1989, the Hessian fly caused an estimated \$4 million per year in damage in South Carolina, and an estimated \$20 million in Georgia from 1988 – 1989 (Buntin et al. 1992, Chapin 2008). Buntin (1999) showed that the Hessian fly can cause an average annual yield loss of 5 – 10% in Georgia, with an estimated 21.1 kg/ha

(0.31 bu/ac) yield loss occurring for each 1% infested tillers in autumn, and an 11.8 kg/ha (0.18 bu/ac) yield loss for each 1% increase in infested tillers in spring. In Oklahoma, regression analysis of winter wheat indicated that yield is reduced by approximately 386 kg/ha (5.74 bu/ac) over the growing season for every one Hessian fly immature per tiller (Alvey 2009).

Management Options

Since the discovery of the Hessian fly in the U.S. in the late 1700's, control practices have included burning and mowing stubble, application of lime, Paris green, Bordeaux mixture, and even kerosene emulsion (Headlee and Parker 1913, Webster 1915, Williamson 1917). However, such approaches were found to be either unsuccessful, unpractical, or potentially dangerous for controlling Hessian flies in large commercial fields. Today common control measures include the use of resistant wheat cultivars, adherence to planting dates that escape early fall infestations (commonly referred to as the “fly-free date”), destruction of volunteer wheat between plantings, and use of insecticidal seed treatments (Foster and Hein 2009, Whitworth et al. 2009, Royer et al. 2015).

Monitoring. Traps utilizing the Hessian fly female sex-pheromone are an effective and efficient method for capturing adult male Hessian fly (Andersson et al. 2009, Schwarting et al. 2015, Knutson et al. 2017). While pheromone traps detect low densities of males in wheat fields, trap captures have not correlated to economically damaging larval infestations in the field and resulting crop damage (Schwarting et al. 2015, Knutson et al. 2017). Lack of correlations between trap catches and economically significant infestations could be due to high egg and neonate larval mortality prior to establishing a feeding site (Knutson et al. 2017). It is also important to note that the pheromone used in the Hessian fly traps only attracts male Hessian

flies (Foster et al. 1991, Andersson et al. 2009), and this may also account for the lack of relationship between trap captures and field infestations.

There is currently no method developed to capture only female Hessian flies. Although the benefit of monitoring for female Hessian flies has not been studied, trapping females of other fly species such as Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), and wheat bulb fly, *Delia coarctata* (Fallen) (Diptera: Anthomyiidae), has been valuable to pest management strategies (Bowden and Jones 1979, Hendrichs 1999, Katsoyannos et al. 1999, Broughton and Rahman 2017). It is important to consider monitoring for adult female Hessian flies in addition to males because female and male movement in the environment is dictated by different factors (Harris and Rose 1990, Harris and Foster 1991), which can result in their movement to different locations in the landscape. Male fly movement after emergence is primarily motivated to find mates. They respond to female sex pheromone to locate females, and mating typically occurs at the site of emergence of females, which begin calling within 10 min of emergence (Bergh et al. 1990, Withers et al. 1997, Anderson et al. 2012). Female movement post-emergence is directed to oviposition site selection (Bergh et al. 1990, 1992), which is governed by multiple environmental factors, as mentioned in the Environmental Dispersal Cues and Dispersal and Flight sections (Harris et al. 1993, Withers and Harris 1997, Withers et al. 1997). Additionally, Withers et al. (1997) estimated that females are capable of moving long distances during oviposition, 660 m² in 2 h in host patches (wheat) and 1,500 m² in 2 h in non-host patches (oat). The capability of females to move long distances during oviposition coupled with the differing factors that drive female and male Hessian fly movement may result in females being present in different locations than males post mating. Therefore it is important to monitor for female Hessian flies in addition to males, as knowing where and when females are present in

the landscape is important for understanding where infestations may occur because females are the primary agent of dispersal through selection of oviposition sites (Harris and Rose 1989).

Thus, monitoring for female Hessian flies deserves further investigation.

Actively sampling and quantifying Hessian fly populations is not commonly adopted as part of the in-season, decision-making process because the nature of most Hessian fly management practices requires they be implemented before infestations have occurred (e.g., resistant cultivars, delayed planting dates, destruction of volunteer wheat). Hessian fly management should be implemented only when the threat of Hessian fly infestation exceeds an economic threshold. However, no economic treatment threshold has been developed for the Hessian fly (Shukle 2008), resulting in management practices being implemented either on a calendar schedule, i.e., fly free date, or in response to historical crop failures for a given production field (with the exception of foliar insecticide application, which has limited application owing to the narrow window of effectiveness and associated cost). Additionally, the preventative nature of most Hessian fly control practices (resistant cultivars, delayed planting, destruction of volunteer wheat, and seed treatments) requires a risk assessment of potential Hessian fly damage weeks in advance of planting to ensure implementation of the practices are justified. The brief window of time (2 – 4 weeks) between the beginning of fall brood emergence (September or October depending on location and weather) and optimum planting dates does not allow producers much time to purchase resistant cultivars, apply seed treatments, or destroy volunteer wheat if Hessian fly is detected in their field prior to planting. However, Hessian fly monitoring can inform producers when Hessian fly begins to emerge from summer aestivation and the level of adult activity in a localized area prior to and after planting (Anderson et al. 2012, Bradford 2014, Schwarting et al. 2015, Knutson et al. 2017). As weather conditions can cause

brood emergence to vary from year to year and additional broods to occur (Drake and Decker 1932, Byers and Gallun 1972), early detection of brood emergence and brood levels prior to planting may aid producers when deciding on a planting date. Additionally, monitoring adult activity can warn producers of the need to check for Hessian fly infestations during the winter and spring months. If infestations of immature Hessian fly threaten crop yield, growers can limit crop inputs, e.g., fertilizer, fungicides, and irrigation, or switch fields from grain production to livestock forage (Knutson et al. 2017).

Plant Resistance. Planting resistant wheat cultivars has long been the most economical and effective control method for Hessian fly (Berzonsky et al. 2003). To date, 34 Hessian fly resistant genes (R) have been identified (Li et al. 2013). Although effective, when widely planted over large areas in consecutive years, a resistant cultivar containing a single resistance gene can rapidly lose effectiveness due to selection for Hessian fly biotypes that overcome the R gene, typically within 6 – 8 years after release (Gould 1986, Ratcliffe et al. 1994, Ratcliffe et al. 2000, Chen et al. 2009b). For example, research testing 21 and 22 R genes found that less than half provided effective protection of wheat against Hessian flies in the Southern U.S. (Cambron et al. 2010, Garces-Carrera et al. 2014). Hessian fly virulence is conditioned by inherited recessive genes (Hatchett and Gallun 1970, Formusoh et al. 1996, Zantoko and Shukle 1997). Hessian flies demonstrate genetic variation in virulence-related genes among individuals (Chen et al. 2010, Zhao et al. 2015), resulting in heterogeneity in fly populations in the field (Ratcliffe et al. 1994, Ratcliffe et al. 1996, Ratcliffe et al. 2000, Chen et al. 2009b, Cambron et al. 2010, Garces-Carrera et al. 2014). As a result of this heterogeneity, the planted resistant wheat cultivar selects for those virulent flies to resistant genes within that wheat cultivar. Thus, damaging outbreaks occur as resistance in the cultivar is lost. Rotating cultivars, each with a different source of

resistance, to vary the R genes planted in subsequent years will help to mitigate loss of cultivar resistance (Gould 1986, Tooker and Frank 2012). Monitoring for virulent biotypes is also important for resistance management, since when these virulent biotypes begin to increase it may be possible to deploy new R genes in the field which are effective against the increasing proportion of virulent biotypes (Chen et al. 2009b, Garces-Carrera et al. 2014). As a result, area wide crop loss can be avoided. In the past, Hessian fly populations virulent to specific R genes were unsystematically named but this is no longer practiced (Ratcliffe et al. 1994). Instead, Hessian fly populations are now named according to their virulence to specific R genes (Chen et al. 2009b, Garces-Carrera et al. 2014). Chen et al. (2009b) defined a gene as highly resistant to a Hessian fly population if $\geq 80\%$ of the plants with that gene are resistant (no larval survival) in a virulence assay, moderately resistant to a population if 50 – 80% of the plants with that gene are resistant, and susceptible to a population if $< 50\%$ of the plants are resistant.

Hessian fly virulence is expressed when saliva from actively feeding larvae cause R genes within the plant to trigger a combination of defensive mechanisms (Subramanyam et al. 2006, Giovanini et al. 2007, Harris et al. 2010, Liu et al. 2010). Some R genes deter additional Hessian fly larval feeding through protein production that specifically targets the larval midgut resulting in inhibition of metabolism and digestion through destruction of midgut microvilli, eventually resulting in larval death due to functional loss of digestion and absorption of nutrients (Giovanini et al. 2007, Liu et al. 2007, Subramanyam et al. 2008, Wu et al. 2008, Shukle et al. 2010). Another protein-encoding gene triggered by the Hessian fly in wheat is *HfrDrd*, which provides a disease resistance-like response against the Hessian fly (Subramanyam et al. 2013). Further advances in understanding the genetic basis for the mechanisms that Hessian fly larvae

use to establish feeding sites could lead to the development of more durable resistance in wheat to Hessian fly (Zhao et al. 2015).

Temperature can also influence the effectiveness of R genes (Garces-Carrera et al. 2014). The resistant genes *H3*, *H5*, *H10*, *H11*, *H12*, and *H18*, have been shown to lose resistance when the temperature rises above a certain threshold (Sosa and Foster 1976, Sosa 1979, Tyler and Hatchett 1983, Ratanatham and Gallun 1986, Buntin et al. 1990b, Cambron et al. 1996). For example, when wheat containing the resistant *H13* gene was exposed to heat stress of 40°C, it became susceptible to avirulent Hessian fly (Chen et al. 2014, Currie et al. 2014). The effect of temperature on some resistant genes can significantly affect the effectiveness of resistant cultivars commonly planted in the U.S. Chen et al. (2014), documented that commonly used cultivars on the Great Plains, such as ‘Bill Brown’, ‘Byrd’, ‘Endurance’, Fuller’, ‘GA-031257-10LE34’, and ‘KS09H19-2-3’, were susceptible at 20°C, but became resistant at lower temperatures. The impact of temperature on resistance will affect Hessian fly management differently across the latitude gradient of the U.S. In Southern states heat stress ($\geq 40^{\circ}\text{C}$) in early fall or late spring is more likely to affect Hessian fly susceptibility than Northern states. Whereas lower temperatures ($\leq 20^{\circ}\text{C}$) in Northern states is more likely to the resistance of fall and spring generations of Hessian flies. Plant resistance is also affected by the order of plant infestation by virulent and avirulent Hessian fly larvae. Infestation of virulent followed by avirulent larvae positively affects larval survival; established virulent larvae induce systemic susceptibility, thus providing refuge for later-infesting avirulent larvae and ultimately resulting in the survival of both (Baluch et al. 2012).

Although virulence assays can identify effective R genes, it is often not known what, if any, R genes are present in commercial wheat cultivars, as is often the case when breeding

programs do not include Hessian fly resistance. Adoption of Hessian fly resistant cultivars is further complicated by the need to consider cultivar yield, disease resistance, and availability to producers in different regions of the U.S. These complications coupled with the effects of temperature, increased survival of avirulent larvae due to virulent larvae infestations, and the potential for R cultivars to lose effectiveness (6 – 8 yr after release) due to regional buildup of resistant biotypes, reinforces the need for more comprehensive Hessian fly integrated pest management (IPM) programs.

Delaying Planting Date. Delayed planting until after a “fly-free date” to escape Hessian fly infestation has been used in the upper Midwest and northern Great Plains states (ranging from North Dakota south to Kansas and extending east to Pennsylvania) since the early 1900s (Whitworth et al. 2009, Tooker 2012, Knodel et al. 2018). A fly-free date indicates when in the late fall adult Hessian fly activity has historically ceased due to cold weather, and thus avoiding infestation by ovipositing females. Fly-free dates are specifically tailored to the environmental conditions of different regions across the country. When the fly-free dates were first documented in the early 1900s, wheat producers held them in high regard. Reports surfaced of farmers secretly plowing under their neighbors’ wheat fields that were planted before the fly-free (Satterthwait 1926). A potential drawback associated with the fly-free date is that later planting dates may increase the risk of winter kill due to cold weather (Campbell et al. 1991, Thiry et al. 2002). Consequently, finding a suitable planting date requires a farmer to weigh the risks of planting too early, which could result in Hessian fly and other key pest infestations, and planting too late, which could result in increased winter kill and reduced forage for fall grazing in Southern states like Texas, Oklahoma, and Kansas (Epplin et al. 1998, Carver et al. 2001). These factors were considered before the optimal fly-free planting dates were recommended (Drake et

al. 1924, Walkden et al. 1944). However, new observations suggest that fly-free dates could be due for revision in some areas of the U.S. For example, Davis et al. (2009) observed Hessian fly adult activity in Kansas later in the fall than previously recorded, and Schwarting (2014) recommended a revision of the fly-free dates in Kansas. It was noted early in the development of the fly-free dates that abiotic conditions, such as unseasonable wet or dry periods, can alter emergence or trigger secondary waves of emergence (Drake and Decker 1932). Even though these variables can affect the effectiveness of the fly-free dates, the technique still remains a good general guideline for a safe planting date to limit Hessian fly infestation in the North-central and Mid-western U.S.

In many of the Southern wheat producing states, such as Georgia, Oklahoma, and Texas, fly-free dates are less effective due to intermittent periods of warm weather that occur throughout the fall and into early winter (Buntin and Chapin 1990, Morgan et al. 2005, Bradford 2014). The periodic warm weather allows adults to emerge and lay eggs, resulting in damaging larval infestations. Even though adult Hessian fly activity does not cease in the Southern states during the fall and winter months, delayed planting based on the number of fall broods and dates of brood emergence can still decrease damage caused by Hessian fly larval feeding (Buntin et al. 1990a, Morgan et al. 2005, Royer and Giles 2009, Knutson et al. 2017). For example, in North Central Texas one to three broods can occur during the fall, and delaying planting until November can help to avoid infestations by the early emerging fall broods (Lidell and Schuster 1990). Although less effective than the fly-free dates observed in the Northern states, delayed planting remains a viable option to reduce the risk of a fall Hessian fly infestation and other economically important pests in Southern states like aphids, fall armyworm (*Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)), armyworm (*Mythimna unipuncta* (Haworth)

(Lepidoptera: Noctuidae)), wheat curl mite (*Aceria tosichella* Keifer (Acari: Eriophyidae), and white grubs (Kimura et al. 2017, McCornack et al. 2017). Monitoring Hessian fly activity with pheromone traps can identify periods of adult emergence in the fall and help refine planting dates.

Destruction of Volunteer Wheat. Volunteer wheat often emerges earlier in the fall than planted wheat, and can host early season development of Hessian fly populations that later lead to higher infestation rates in the subsequently planted wheat crop (Buntin et al. 1991). Much like fly-free dates, destruction of volunteer wheat has been recommended as an important control measure since the early 1900s (Headlee and Parker 1913). Despite the widespread practice of controlling volunteer wheat, surprisingly little research has been published on the subject. Parks (1917) determined that the presence of volunteer wheat before planting enhanced Hessian fly infestations and negated the benefit of planting after the fly-free date. Buntin et al. (1991) demonstrated that destruction of volunteer wheat prior to wheat planting reduced the risk of Hessian fly infestation. Timing of volunteer wheat destruction is important as it has been recommended to occur at least two weeks before germination of the planted crop for the most effective results (Whitworth et al. 2009).

The value of tillage to destroy Hessian fly puparia in wheat residue varies. Studies of no-till systems compared to conventional tillage have mixed results (Chapin et al. 1992, Zeiss et al. 1993b, Del Conte et al. 2005). In these studies, successive tillage regimens and the depth that the residue was buried influenced fly survival and subsequent infestations in the wheat crop (Chapin et al. 1992, Zeiss et al. 1993b). Thus, tillage practices that sufficiently bury stubble (9 – 11 cm or 3.5 – 4.3 inches) and avoid repetitive tillage that can resurface buried flaxseed remains a valid method to destroy volunteer wheat to kill Hessian fly flaxseeds (Chapin et al.

1992, Zeiss et al. 1993b, Flanders et al. 2013). However, no-till systems designed to reduce soil erosion, improve soil structure, and increase organic matter content will likely continue to increase in popularity (Derpsch et al. 2010). Suppression of Hessian fly infested volunteer wheat through tillage will likely become a greater challenge for these producers, and thereby become a less utilized management tool in the future. Herbicides are an effective alternative to tillage for control of volunteer wheat to break the ‘green-bridge’ and control pest insects like the Hessian fly (Bell et al. 2016). However, while herbicides will control volunteer wheat, they will not reduce the presence of wheat stubble from the previous crop that may harbor flaxseeds.

Insecticides. Systemic seed treatment products labeled for Hessian fly may control fall infestations of Hessian flies in winter wheat; however, they provide little to no protection from spring infestations (Wilde et al. 2001). In the Northern wheat producing states, seed treatments provide temporary control, ~30 days, which can be effective if there is only one fall generation of Hessian fly (Wilde et al. 2001, Whitworth et al. 2009). In Southern states such as Texas and Oklahoma, the 20 – 30 days of protection provided by seed treatments after germination again control the first brood of flies infesting wheat but are not effective against subsequent broods (Morgan et al. 2005, Royer et al. 2015). Seed treatments also control other early season insect pests (aphids) but the cost of investing in a preventative seed treatment for Hessian fly alone often cannot be justified. Seed treatments can be important when there is a history of high Hessian fly infestation and/or resistant cultivars are not available, and other management strategies, i.e., delayed planting and destruction of volunteer wheat, have been implemented if possible (VanDuyn et al. 2003, Morgan et al. 2005, Flanders et al. 2013).

Foliar-applied insecticides, typically pyrethroids, are targeted to control adults and neonate larvae before they reach the leaf sheath where they are protected from the treatment.

Treatments are most effective when applied when seedling plants have 2 – 3 leaves. In the Southeastern U.S., foliar applied insecticides can be considered when at least three of the following five conditions are met: 1) the current wheat crop was planted directly in or within 400 yards (365.8 m) of a wheat field of the previous year, 2) a resistant cultivar was not planted in the current field, 3) neonicotinoid seed treatment was not applied to the current field, 4) yield loss due to Hessian flies has occurred in nearby fields in previous years, 5) Hessian fly eggs are found on the wheat leaves of the current crop (VanDuyn et al. 2003, Flanders et al. 2013). However, foliar applications are only effective if applied when adults are laying eggs, eggs are present on leaves, and before larvae have established in the stems (VanDuyn et al. 2003, Buntin 2007) and because multiple broods occur throughout the growing season, multiple foliar insecticide applications would be necessary. The limited window of effectiveness, inconsistent infestation rate of Hessian fly, difficult timing of foliar applications, lack of efficient sampling methods and economic treatment threshold, and associated costs of multiple applications are the main reasons why foliar-applied insecticides remain one of the lesser-used management options (VanDuyn et al. 2003, Alvey 2009, Knutson et al. 2017).

Natural Enemies. Many species of Hymenopteran parasitoids (wasps) attack the Hessian fly. Gahan (1933) described 41 species of Hessian fly parasitoid wasps in North America and Europe. Most of these parasitoids belong to the superfamily Chalcidoidea, which attack the puparia of the spring generation of Hessian fly; however, five parasitoids in the family Platygasteridae attack the egg stage, which includes *Platygaster hiemalis* Forbes (Hymenoptera: Platygasteridae) that parasitizes fall generations (Gahan 1933). Although the composition of parasitoid communities vary significantly among regions in the U.S., three species (*P. hiemalis*, *Homoporus destructor* (Say) (Hymenoptera: Pteromalidae), and *Eupelmus allynii* French

(Hymenoptera: Eupelmidae)) are widespread and are considered valuable parasitoids (Rockwood and Reeher 1933, Hill 1953, Schuster and Lidell 1990). Parasitoids cause significant mortality to Hessian fly populations, as high as 55%, 87%, and 98% parasitism observed in fields in Georgia, Texas, and Washington, respectively (Morrill 1982, Pike et al. 1983, Schuster and Lidell 1990). However, parasitism rates vary significantly between fields, generations, time of year, host density, and geographic location (Hill et al. 1939, Pike et al. 1983, Prestidge 1992, Wise 2007); owing to inconsistencies in their life history (egg vs. puparia parasitoid, or attacking fall vs. spring broods) and variation in population composition across regions. Inconsistent Hessian fly parasitism was highlighted by Schuster and Lidell (1990), who observed parasitism rates varied significantly from 0 – 87% in Texas wheat fields, with parasitism of fall Hessian fly generations rare compared to spring parasitism rates, and parasitoid species fluctuating widely between counties and years.

Pupal parasitoids result in Hessian fly mortality only after the larva has damaged wheat plants. When populations of Hessian flies in the Southern states break dormancy in the fall, populations can still rapidly increase during the two generations that can be completed before winter dormancy begins (Schuster and Lidell 1990, Knutson et al. 2002). Thus, in states where parasitoids are only active in late spring, parasitism does not protect the current crop, but spring parasitoids can reduce the number of Hessian fly entering summer aestivation (Schuster and Lidell 1990). This makes pupal parasitoids important for the protection of resistant cultivars, as parasitoids are capable of finding and parasitizing low densities of Hessian fly puparia (Knutson et al. 2002), which represent virulent biotypes that survive on resistant cultivars. The egg-larval parasitoid *P. hiemalis*, which attacks fall broods of Hessian fly, can significantly enhance the effectiveness of resistant cultivars, reducing Hessian fly larval survival to 2.5% (a 42% reduction

compared to larval survival on solely the resistant cultivar) (Chen et al. 1991). The interaction between resistance and *P. hiemalis* was most effective when a cultivar of intermediate resistance was combined with the parasitoid (Chen et al. 1991). The ability of parasitoids to enhance resistant cultivars through increased Hessian fly mortality demonstrates the importance of conserving parasitism as a management technique. Practices that conserve Hessian fly parasitoids have not been investigated and more research is needed to understand how to increase Hessian fly parasitoid populations and improve the consistency of parasitism rates. Furthermore, understanding Hessian fly distribution (both adult and pupae) in commercial wheat fields may lead to the development of parasitoid release programs to manage Hessian fly populations.

Integrated Management

Although the aforementioned control measures can impact Hessian fly populations if applied individually, they can be more effective at reducing Hessian fly losses when used in combination as part of an IPM program (Buntin et al. 1991, Chen et al. 1991, Buntin et al. 1992). Buntin et al. (1992) showed that a systemic insecticide combined with delayed planting is an economically effective Hessian fly management strategy when high-yielding resistant cultivars are not available. Also, parasitoids can enhance the control of Hessian fly in fields planted with wheat cultivars of intermediate resistance (Chen et al. 1991). Not only are Hessian fly management practices often compatible with each other, but they can be integrated with management plans for other insect pests of wheat. For instance, delayed planting not only reduces risk of Hessian fly infestation, but it also reduces the risk of other economically important pests in Southern states like aphids, fall armyworm (*S. frugiperda*), armyworm (*M. unipuncta*), wheat curl mite (*A. tosichella*), and white grubs (Kimura et al. 2017, McCornack et al. 2017). However, not all wheat management practices are compatible with all forms of

Hessian fly management. Namely, no-till soil conservation does not comply with disk harrowing before planting to bury volunteer wheat/wheat stubble harboring flaxseed (Chapin et al. 1992). This highlights the need to improve upon the current Hessian fly monitoring technique (pheromone trap) to correlate trap capture with infestations, so that producers can select appropriate Hessian fly management practices that integrate with wheat field management (e.g., soil, insect, and weed management) or limit crop inputs (e.g., fertilizer, fungicides, and irrigation, or switch fields from grain production to livestock forage) (Knutson et al. 2017). For instance, a correlation between trap capture and infestation level prior to planting could help determine planting dates or application of insecticide treatments, while also warning producers to continue monitoring throughout the fall and spring months to assess infestation levels and mitigate crop inputs appropriately.

Conclusion

Since the introduction of the Hessian fly in the late 1700s (Pauly 2002), this pest is responsible for significant economic damage in many of the wheat production regions of the U.S. (Smiley et al. 2004, Watson 2005, Alvey 2009). As a result of the potential yield loss associated with Hessian fly larval feeding, multiple control tactics have been researched and developed into an IPM program that provides options for producers to integrate Hessian fly management with wheat production and control of other pest insects. Integrated Hessian fly management recommendations include:

- Incorporate resistant cultivars, adherence to optimum planting dates (i.e., “fly-free dates” in Upper Midwest and Northern Great Plains states or delayed planting in Southern states to avoid the first fall brood), destruction of volunteer wheat two weeks prior to planting, natural enemies, and insecticides to manage Hessian fly infestations.

- Foliar applied insecticides should only be considered when at least three of the following five conditions are met: 1) the current wheat crop was planted directly in or within 400 yards of a wheat field of the previous year, 2) a resistant cultivar was not planted in the current field, 3) neonicotinoid seed treatment was not applied to the current field, 4) yield loss due to Hessian flies has occurred in nearby fields in previous years, 5) Hessian fly eggs are found on the wheat leaves of the current crop (VanDuyn et al. 2003, Flanders et al. 2013). Insecticide application strictly for Hessian fly control is limited.
- Systemic seed treatments provide temporary control (~30 days) of fall infestations of Hessian flies in winter wheat; however, they provide little to no protection from spring infestations (Wilde et al. 2001).
- Resistant wheat cultivars have long been the most economical and effective control method (Berzonsky et al. 2003), and cultivars should be rotated, each with a different source of resistance, to vary the R genes planted in subsequent years to help mitigate loss of cultivar resistance (Gould 1986, Tooker and Frank 2012).
- If cultivars with only intermediate resistance are available, the fall parasitoid *P. hiemalis* can significantly enhance the effectiveness of those cultivars (Chen et al. 1991).
- Cultivar selection must balance the level of Hessian fly resistance with cultivar yield, disease resistance, and availability to producers in different regions of the U.S.
- No single technique should be considered as a “silver bullet” for management of Hessian fly in wheat. Instead, management practices should be used in conjugation when feasible and economically beneficial (e.g., a combination of volunteer wheat destruction with delayed planting, or integration of systemic insecticide with delayed plant) (Buntin et al. 1991, Buntin et al. 1992).

- Consider incorporating pheromone traps into the implementation of certain Hessian fly IPM tactics (i.e., delayed planting, systemic seed treatment), as these traps can accurately and efficiently define periods of male Hessian fly activity.
- Hessian fly management tactics, i.e., delayed planting, destruction of volunteer wheat, and insecticides, should be integrated with wheat production practices (e.g., no-till) and additional pest management programs to reduce risk of other economically important wheat pests (e.g., aphids, fall armyworm, armyworm, wheat curl mite, and white grubs in Southern states) (Kimura et al. 2017, McCornack et al. 2017).

Future research on these management practices along with the development of sampling techniques, time to sample, and treatment thresholds can advance the implementation of the various methods of Hessian fly IPM. Especially research focusing on the genetic mechanisms involved in the establishment a feeding site by Hessian fly larvae, which can lead to the development of new resistant cultivars. Additionally, a better understanding of egg and neonate mortality and weather conditions could improve the relationship between pheromone trap data and subsequent field infestations by Hessian fly larvae by understanding when high trap capture is mitigated by high larval mortality due to adverse weather. Strategies examining attractants of female Hessian flies may improve correlations between trap catches and field infestations by increasing the knowledge of female distribution in the landscape; thus, monitoring for females should also be the focus of future studies.

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Tables and Figures

Figure 1.1. Adult Hessian fly. A) Male. B) Male genitalia. C) Female. D) Female genitalia.
(Photo credits (all): Alan Burke)

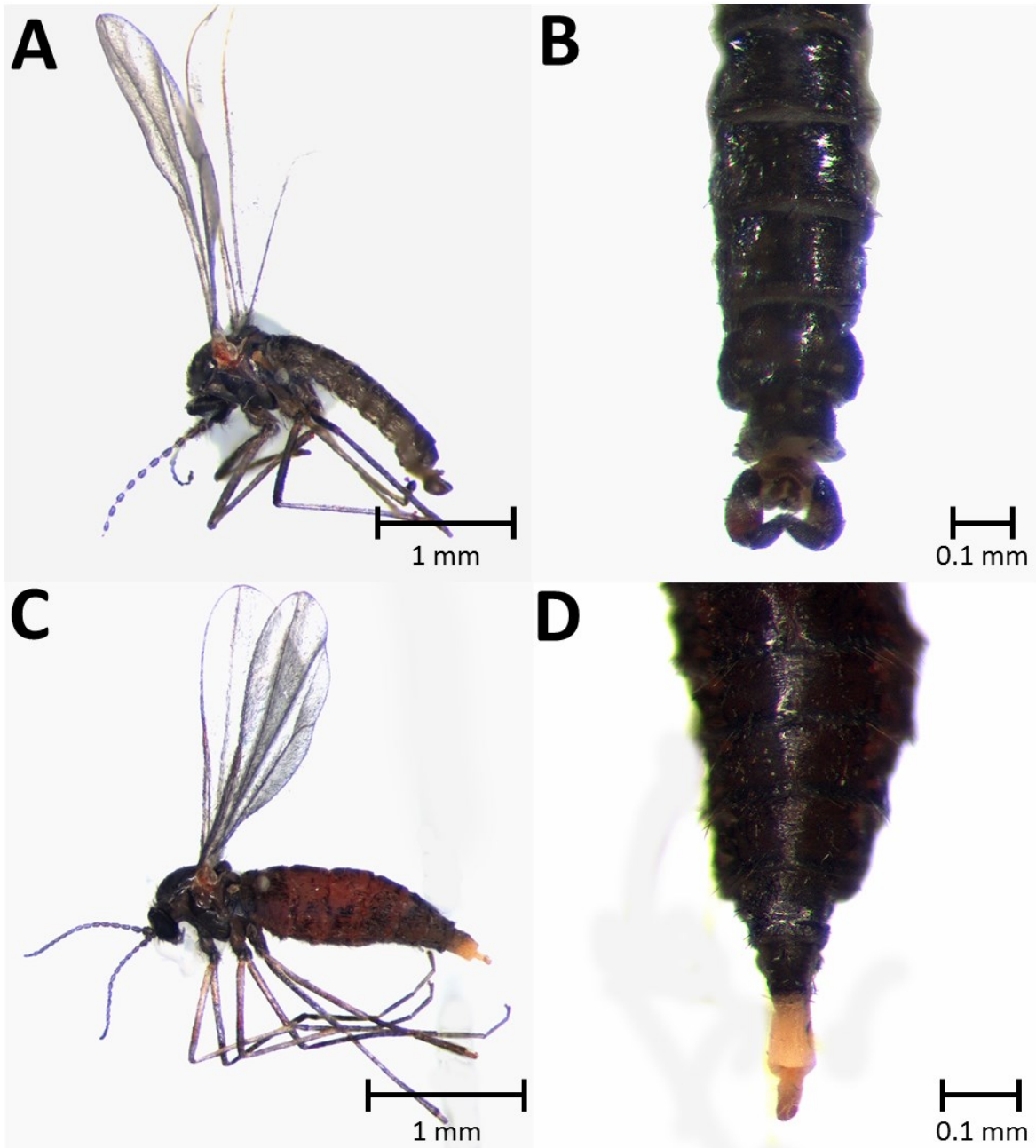


Figure 1.2. Immature Hessian fly life stages. All photos are of wheat infested with Hessian fly.

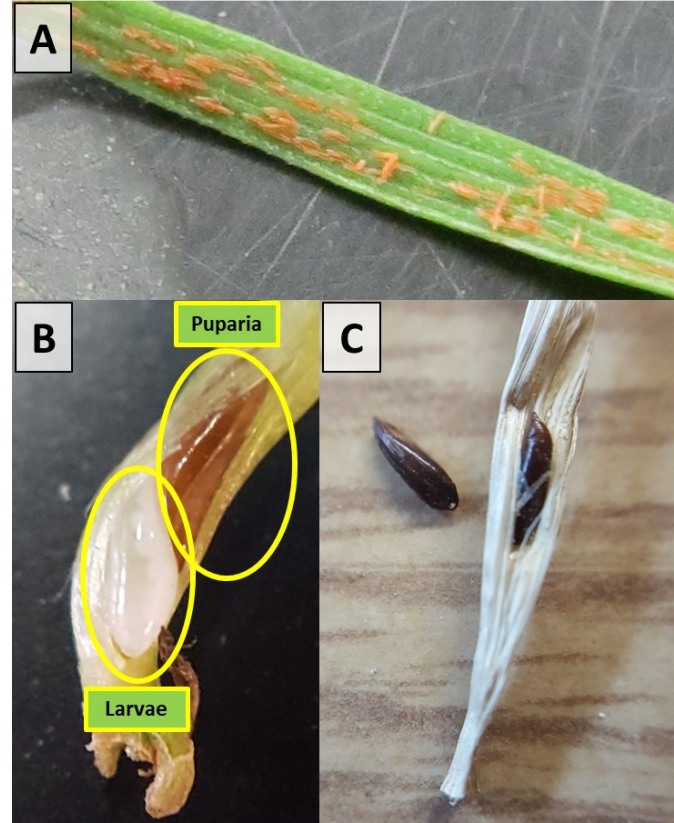


Figure 1.3. Typical damage to wheat due to feeding by Hessian fly larvae. A) Stunted growth of Hessian fly infested wheat compared to uninfested wheat (left) (Photo credit: Tom A. Royer, Oklahoma State University). B) Lodged wheat due to Hessian fly infestation (Photo credit: Tom A. Royer, Oklahoma State University). C) Wheat variety study containing Hessian fly resistant varieties (green plots) and susceptible varieties (brown plots). D) Dark, blue-green, foliage of Hessian fly infested wheat compared to uninfested wheat (left).



Chapter 2 - Introduction

Early detection of insect pests is a time-sensitive process that requires development of monitoring approaches for execution of incursion mitigation and IPM protocols. Insect monitoring can demand a significant number of trained personnel to detect targeted species. Passive sampling offers a cost-effective solution to handling the staggering task of monitoring for pests. However, given the number of different monitoring methods, from specific (e.g., pheromone lures) to general (e.g., sticky cards), there is a need to develop protocols for deploying traps to effectively and efficiently monitor for a multitude of potential pests. More specifically, sensor technologies (light, auditory, pheromone, etc.), placement of monitoring tools, and density of those tools may vary across taxonomic groups. More information is required to link design to efficiency. It is imperative to address trap efficacy, as these outcomes are directly transferable to known and potential insect monitoring problems.

The Hessian fly is a suitable study organism to examine sensor technologies and placement strategies, owing to the behavioral traits of the insect. Hessian flies exhibit non-random movement toward several cues, including pheromone, plant extract, color, and spatial configuration (Harris and Rose 1990, Withers and Harris 1996, Anderson et al. 2012). Specifically, female taxis during oviposition utilizes chemical, visual, and tactile characteristics of host plants (Harris and Rose 1990), while male movement is directed toward the female sex-pheromone (McKay and Hatchett 1984, Andersson et al. 2009). However, males may also respond to similar habitat cues as females as a means to locate females, but this has not been reported in the literature. Components of the female sex-pheromone have been identified and developed into a synthetic lure, but captures of male Hessian flies in traps utilizing the pheromone lure do not correlate to field infestations (Schwartz et al. 2015, Knutson et al.

2017). A better monitoring approach is needed to correlate trap captures with field infestations. Given that female Hessian flies are the primary driver of larval dispersal through oviposition site selection (Harris and Rose 1989), a better understanding of both sexes behavior toward the multiple known attractants is required to improve trap design. The hierarchy of female attraction to visual cues during oviposition before chemical and tactile (Harris and Rose 1990) lends credence to further investigate new visual sensor technologies as monitoring techniques for Hessian flies. Furthermore, it is not well understood where economically significant outbreaks of Hessian fly are more likely to occur. This hinders trap placement and deployment strategies, as it is unknown where to place traps in the landscape to monitor for adult activity. Several environmental characteristics affect female dispersal in the environment (e.g., wind speed and direction, composition of plant patches, and proximity to host reservoirs) (Withers and Harris 1996, 1997, Withers et al. 1997). Examining Hessian fly distribution in the field and the environmental factors that may affect their distribution can increase the efficiency of trap placement.

This dissertation will examine Hessian fly monitoring strategies through a series of laboratory experiments and initial efficacy trials, and supported with field evaluations to determine trap efficiency. This will lead to the accumulation knowledge of Hessian fly behavior toward new sensor technologies (light emitting diodes (LEDs) and laser displays), strategies to utilize these technologies for Hessian fly monitoring, and deployment of the strategies. Laboratory and field efficacy trials will be conducted to show viability of sensor technologies as a Hessian fly monitoring method. It is imperative that we demonstrate trap efficacy under field conditions prior to making monitoring recommendations to producers.

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Chapter 3 - Hessian Fly, *Mayetiola destructor* (Diptera: Cecidomyiidae), Attraction to Different Wavelengths and Intensities of Light Emitting Diodes in the Laboratory

Introduction

The Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), is a significant pest in wheat production systems around the world (Stuart et al. 2012, CABI 2016). This includes the United States, where it has the potential to cause economic injury in many of the wheat producing regions (Smiley et al. 2004, Watson 2005, Alvey 2009). Not only is Hessian fly a significant wheat pest, it is a model system to study gene-for-gene interactions, which are of paramount interest for plant pathology and resistant cultivar development (Stuart et al. 2012, Harris et al. 2015), and is one of the few insect herbivores with a sequenced genome (Zhao et al. 2015). The ability to monitor and sample for adult Hessian fly, both male and female, in the environment would not only improve the study of Hessian fly population genomics. Sampling both sexes would also aid in quantifying the geographic distribution, understanding changes in phenology and population dynamics of this pest.

As described by Harris and Foster (1999), Hessian flies exhibit characteristics that make many members of the Cecidomyiidae family difficult to detect, which include small life stages, synchronized eclosion and flight activity, short adult life span, and sheltered larval stage. Also, damage to the host plant is often subtle, resulting in delayed detection within cropping systems and allowing time for populations to build and become a problem (Harris and Foster 1999). Yet the Hessian fly has an advantage over other Cecidomyiids in terms of monitoring, which is the identification of female Hessian fly sex pheromone components. The first component of the pheromone was isolated and identified in the early 1990s (Foster et al. 1991, Harris and Foster

1991, Millar et al. 1991); however, it took over 15 years before the other four components were identified (Andersson et al. 2009). Field methods for Hessian fly trapping have now been developed using the identified sex pheromone components (Anderson et al. 2012). While traps utilizing the Hessian fly sex pheromone are effective at capturing males, current trapping methods only utilize a female sex pheromone and do not attract and capture females. Given that female Hessian flies are important for both the previously mentioned genetic studies and pest management aspects, as females select oviposition sites which eventually cause crop damage, it is important to monitor for female Hessian flies as well as the males.

Female Hessian fly taxis in relation to oviposition has been studied extensively (Morrill 1982, Bergh et al. 1990, Withers and Harris 1996, 1997). During oviposition host selection, female Hessian flies utilize chemical, visual, and tactile characteristics of the host plant to determine suitability of the host (Harris and Rose 1990). Of these plant characteristics, visual cues, which include yellow-green light, vertical contours, and a larger overall area, are most important for female orientation to and landing on host plants (Harris et al. 1993). Specifically, flight responses were both wavelength and intensity dependent, with wavelengths in the region of 530 – 560 nm stimulating the females to approach and contact plants/targets (Harris et al. 1993). This suggests that visual targets of specific wavelengths of light could be used to monitor female Hessian flies using visual cues, as traps with species specific attractants have been developed for other insect species (Yee 2013, Poland and McCullough 2014, Tang et al. 2016). However, colored paper targets were used in the study by Harris et al. (1993) to examine female Hessian fly response to colors, and unfortunately paper targets are a less controlled procedure for testing color responses, as the reflected light from paper targets has the potential to have a broad

spectrum reflectance pattern, owing to the variance of wavelengths from light sources and reflectance off of targets.

Light-emitting diodes (LEDs) offer many advantages to study insect phototaxis, one advantage being the capability to emit light within a desired narrow spectral range and intensity, which offers a more controlled to examine insect response to specific wavelengths. For example, Snyder et al. (2016) used narrow spectrum LEDs in a series of laboratory bioassays to study light spectra preferences of *Culicoides sonorensis* Wirth and Jones (Diptera: Ceratopogonidae). In addition to examining wavelength-specific responses, LEDs have also been successfully incorporated into traps to capture insects (Bishop et al. 2004b, Cohnstaedt et al. 2008, Duehl et al. 2011). Just as LEDs aided in the study of wavelength-specific response and improved trap capture of other insects, LEDs could offer similar advantages to study Hessian fly phototaxis or be used to enhance current monitoring techniques.

The objective of this study was to examine adult Hessian fly attraction to various wavelengths and intensities of light emitted by LEDs. To assess Hessian fly attraction to LEDs, three separate bioassays were conducted. The first bioassay examined Hessian fly attraction to different hues of color emitted by LEDs. The second bioassay examined Hessian fly attraction to wavelengths of light within the green spectrum, as green was the most attractive color in the first bioassay. The last bioassay examined Hessian fly attraction to different intensities of light emitted by LEDs. Our hypothesis, based on Harris et al. (1993), was that Hessian flies would be attracted to the highest intensity LEDs, emitting a narrow wavelength range in the green spectrum between 530 nm and 560 nm.

Materials and Methods

Insect Rearing. All Hessian flies used in this study were biotype Scott-KS-GH-05, which were originally collected from wheat fields in Scott County, KS in 2005 - 2008 (Chen et al. 2009b, Chen et al. 2009a). All life stages of Hessian fly colony were completed on susceptible wheat seedlings (Karl 92) in the greenhouse over a 29 – 35 d generation period (Tan et al. 2013). The colony was kept at 22°C and a photoperiod of 16:8 (light:dark) h. By continuously rearing the colony on wheat, female Hessian flies used natural host cues to select suitable oviposition sites. This has important implications for our study's examination of female attraction to light wavelengths, as visual attraction to wheat during oviposition site selection influenced our hypothesis. Hessian fly adults used in the bioassays were all newly eclosed the previous late afternoon and evening (just males emerge (Bergh et al. 1990)) or the early morning (both females and a small number of males emerge (Bergh et al. 1990)) of each bioassay replicate.

Bioassays. Three incremental bioassays were conducted to assess male and female Hessian fly attraction to LEDs using 1) a broad spectrum of wavelengths (ranging from 465 nm to 630 nm), 2) narrow wavelengths based on broad-spectrum bioassay (502 nm to 565 nm), and 3) different-intensities (4 W/m² to 16 W/m²) of the most preferred wavelength, which was 525 nm (see Results). The wavelength (nm) and intensity (W/m²) of light emitted from LEDs was measured with a StellarNet light spectrometer (BLACK Comet C-SR-25; Stellar Net, Inc., Tampa, FL, U.S.A.). LEDs used to test Hessian fly attraction were 5-mm round through hole bulbs. In all three LED bioassays, newly eclosed Hessian flies were removed from the colony at 0700 h, and transferred to light assay arena at 0730 h for the start of each bioassay. The number of individuals used per replicate throughout each bioassay varied due to the availability of newly eclosed flies emerging from the lab colony on the morning of each replicate.

Light arena. All three bioassays used the same light arena to examine Hessian fly attraction to various LED wavelengths and intensities and followed protocols described by Snyder et al. (2016). The light arena consisted of four connected, half-circles of Plexiglass that formed a cloverleaf shape with outside dimensions measuring 25 × 25 cm (Fig 3.1). Positioned at the apex of each half circle was a collection cup that contained a LED of either different color hue, wavelength within the green spectrum, or intensity, depending on the bioassay conducted. LEDs were randomly assigned to new collection cups for each replicate in each bioassay. Flies were released from the center of the arena and allowed to acclimate to the light arena environment for 1 h before turning on LEDs. Flies were never found to have moved into the collection cups during the 1 h acclimation period before LEDs were turned on. The arena was housed inside a cage during each bioassay to block ambient light from entering the arena, thus the LEDs used during each bioassay were the only light sources in the arena. Flies remained in the light arena for 23 h to allow sufficient time for individuals to make a choice. Bioassay replicates ended after 23 h due to Hessian flies short adult life span, 1 – 4 days, (Bergh et al. 1990), which resulted in the death of most of the flies released into the light arena within the 23 h. Flies that made a LED choice remained in the collection cup due to attraction to the LED and the long entrance tube which made it difficult for flies to leave collection cups. After 23 h, collection cups were removed, and the number of individuals in each cup was counted along with the number that remained in the center of the light arena (i.e., flies that did not choose an LED). The sex of each fly placed in the arena was then verified to ensure all flies placed in the arena were the intended sex. This procedure confirmed that all flies utilized in their respective bioassays were of the intended sex.

Broad-spectrum bioassay. To examine Hessian fly preference for different wavelengths, male and female flies were released simultaneously in the center of the light arena during each replicate (n = 8). Sex ratios were similar in all eight replicates ($\chi^2 = 7.00$; $P = 0.43$). Flies used per replicate ranged from 36 – 49, except for one replicate that contained 100 flies. The colors emitted by the LEDs were blue (465 – 480 nm) (Part #: C503B-BCS-CV0Z0461, Cree, Inc. Durham, North Carolina), green (520 – 535 nm) (Part #: C503B-GCS-CY0C0791, Cree, Inc. Durham, North Carolina), amber (584 – 596 nm) (Part #: C503B-ACS-CX0Z0251, Cree, Inc. Durham, North Carolina), and red (618 – 630 nm) (Part #: C503B-RCS-CW0Z0AA1, Cree, Inc. Durham, North Carolina). The LEDs were selected based on wavelength ranges that did not overlap and colors that covered a wide range of the visible spectrum (465 to 630 nm). Also, these LEDs are commercially available at low cost, which is an important consideration when developing insect traps. Green and amber LEDs were specifically included in the bioassay because of the work by Harris et al. (1993), which demonstrated female orientation to targets reflecting wavelengths from 530 to 560 nm. Each LED was set to a similar intensity according to observations by the authors; however, we were unable to confirm LED intensities were the same for each LED before the beginning of this bioassay due to a malfunctioning spectrophotometer. Upon completion of all replicates of this bioassay, we were able to confirm the intensities of the LEDs used in this bioassay were 2.9 W/m² for blue, 2.7 W/m² for green, 2.8 W/m² for amber, and 3.2 W/m² for red.

Green spectrum bioassay. Results from the broad-spectrum bioassay demonstrated that both sexes of Hessian fly chose the green LED significantly more than any other color LED (see Results). Therefore, we conducted a second bioassay to examine Hessian fly attraction to wavelengths within the green spectrum for commercially available LEDs, which ranged from

502 – 565 nm. Due to the potential of Hessian fly males responding to females captured in collection cups during the bioassay, male and female flies were released in the light arena separately, with 8 and 7 replicates run for each sex respectively. The number of male and female flies used per replicate ranged between 5 – 18 and 30 – 40 flies, respectively. The wavelengths of light emitted by the LEDs were 502 nm (Part #: SSL-LX5093UEGC, Lumex, Hsin Chu County, Taiwan), 525 nm (Part #: LTL2T3TGK6, Lite-On, Inc., Taipei, Taiwan), 565 nm (Part #: WP7113MGC, Kingbright, Taipei, Taiwan), and white (Part #: SLA560WBC7T3, Rohm Semiconductor Co. Ltd., Kyoto, Japan). Each LED was set to the same intensity (12.5 W/m²). The white LED was used in this study to serve as a positive control, representing a light source emitting wavelengths across the visible spectrum, which ranged from 380 to 750 nm.

Light intensity bioassay. Results from the previous bioassay determined the targeted wavelength used in this study, which ranged from 520 to 525 nm (see Results). To examine Hessian fly attraction to emitted light of different intensities, male and female flies were released in the light arena separately with 7 and 8 replicates run for each sex respectively. The number of male and female flies used per replicate ranged between 36 – 39 and 36 – 40, respectively. The intensities of light emitted by the LEDs were 4 W/m² (Part #: LTL2T3TGK6, Lite-On, Inc., Taipei, Taiwan), 8 W/m² (Part #: LTL2T3TGK6, Lite-On, Inc., Taipei, Taiwan), 12 W/m² (Part #: C503B-GCS-CY0C0791, Cree, Inc., Durham, North Carolina), and 16 W/m² (Part #: C503B-GCS-CY0C0791, Cree, Inc., Durham, North Carolina). Each LED was set to a similar light wavelength (520 – 525 nm). Wavelength varied slightly due to the need to produce a wide range of light intensities for the test and the capability of available LEDs to produce the intended intensities.

Data Analysis. Due to unequal sample size between replicates, the percentage of flies that chose each LED treatment within a replicate was used to standardize results between replicates during data analysis. Percent fly response for each treatment was calculated using the total number of flies that responded to the treatments, non-responsive flies were not factored into the percent fly response per treatment. Thus, percent fly response was calculated by dividing the number of flies collected from each collection cup by the total responsive flies. The percent Hessian fly response to LED treatments was compared using one-way analysis of variance (ANOVA). Specifically proc GLIMMIX, an analysis matrix that accounts for non-normality, was used as LED choice data were non-normally distributed. Means were separated using Tukey–Kramer test (SAS version 9.4). Statistical significance for P -value was set at $\alpha = 0.05$.

Results

Broad-Spectrum Bioassay. Hessian fly response to colored light emitted from LEDs was significantly different for both females ($F_{3, 28} = 44.41$; $P \leq 0.01$) (Fig 3.2A) and males ($F_{3, 28} = 3.97$; $P = 0.02$) (Fig 3.2D). Female Hessian flies responded to green LEDs 1.8 times more than the blue LED, the color with the seconded highest female choice. Female response to the amber and red LEDs was significantly lower, 33.0 and 39.1 times respectively, than the response to green. Male Hessian flies exhibited a similar pattern to female response toward LEDs. Male flies chose the green LED significantly more than amber and red but not blue. The response of Hessian flies to any of the four LED choices was significantly different between the sexes ($F_{1, 14} = 17.93$; $P \leq 0.01$) (Fig 3.3A); where male response to the LEDs was only $19.0 \pm 4.7\%$, as opposed to $55.4 \pm 7.2\%$ female response to the LEDs, meaning a large percentage of both male and female Hessian flies did not respond to the LEDs, or 81.0% and 44.6% respectively.

Green-Spectrum Bioassay. Hessian fly response to different wavelengths was significantly different for both females ($F_{3, 24} = 17.71$; $P \leq 0.01$) (Fig 3.2B) and males ($F_{3, 28} = 5.64$; $P \leq 0.01$) (Fig 3.2E). Female flies chose the wavelengths 502 nm and 525 nm significantly more than the other wavelengths of 565 nm and the white control LED. Female Hessian flies responded to LEDs in the lower regions of the green spectrum (502 and 525 nm) 22.82 times more often than the upper region of the green spectrum (565 nm). Male Hessian flies chose the wavelengths 502 nm and 525 nm $37.9\% \pm 14.8$ and $46.4\% \pm 14.8$ respectively, but showed no statistical preference between LEDs of the lower region of the green spectrum (502 nm and 525 nm) and 565 nm or between 565 nm and the white LED. The response of Hessian flies to any of the four LED choices was significantly different between the sexes ($F_{1, 13} = 13.48$; $P \leq 0.01$) (Fig 3.3B); where male response to the LEDs was $35.4 \pm 4.9\%$, as opposed to $66.2 \pm 7.0\%$ female response to the LEDs, resulting in 64.6% of males and 33.8% of females not responding to the LEDs.

Light-Intensity Bioassay. Hessian fly response to different LED intensities was significantly different for both females ($F_{3, 28} = 21.00$; $P \leq 0.01$) (Fig 3.2C) and males ($F_{3, 24} = 30.75$; $P \leq 0.01$) (Fig 3.2F). The highest LED intensity, 16 W/m², was chosen significantly more than any of the other intensities by both females and males, producing a response 1.38 and 2.38 times higher than all other intensities combined for female and male flies, respectively. Female and male Hessian flies then chose 12 W/m² significantly more than 8 W/m² or 4 W/m². The response of Hessian flies to any LED was significantly different between the sexes ($F_{1, 13} = 27.72$; $P \leq 0.01$) (Fig 3.3C). Only $51.5\% \pm 7.6$ of the male Hessian flies responded to LEDs, as opposed to $90.7\% \pm 2.2$ of the female flies responding to the LEDs, resulting in a small percentage of nonresponding female, 9.3%, compared to males, 48.5%.

Discussion

This series of bioassays demonstrates response by both sexes of Hessian fly to emitted light from LEDs. These bioassays also show a significant effect of LED wavelength and intensity on Hessian fly response. Our results are similar to other studies that examined insect attraction to LEDs, which demonstrated that both LED wavelength and intensity can affect insect attraction (Mellor and Hamilton 2003, Burkett and Butler 2005). For example, Snyder et al. (2016) reported the biting midge *Culicoides sonorensis*, Wirth and Jones (Diptera: Ceratopogonidae), was attracted to LEDs in the UV spectra (390 nm) significantly more than blue (460 nm), green (560 nm), and red (640 nm) LEDs, and UV LEDs (395 nm) set to higher intensities attracted significantly more midges than lower intensity LEDs of the same wavelength. In prior laboratory studies female Hessian flies have demonstrated similar abilities to differentiate wavelengths. In an oviposition preference bioassay, female Hessian flies were attracted to reflected light in the 530 – 560 nm range of the green spectrum, and the intensity of reflected light also affected female preference (Harris et al. 1993). However, the results of our bioassays differ slightly from the results Harris et al. (1993) and consequently our hypothesis.

Our results demonstrated female preference for LEDs emitting light in the lower region of the green spectrum (502 nm and 525 nm) instead of the hypothesized range of 530 – 560 nm (yellow-green) based on Harris et al. (1993). The Hessian fly is not alone in this phenomenon, the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), has also shown a discrepancy in response between LEDs and reflected light from targets, in which whiteflies preferred green LED traps over 50% more than the traditional monitoring method of yellow sticky cards (Stukenberg et al. 2015). The discrepancy between our results, which used LEDs, and the study by Harris et al. (1993), which examined reflected light off of paper targets,

could be due to differences between emitted versus reflected light. One important difference between emitted light from LEDs and reflected light is the spectral range produced from each feature. Reflected light can potentially have a broad spectrum reflectance pattern, even though objects reflecting light appear as one color to the human eye. For example, yellow sticky cards used to attract and trap insects often have high reflective intensity in the green spectrum and a low reflective intensity in the blue spectrum in addition to reflectance in the yellow spectrum (Vernon and Gillespie 1990, Petrice and Haack 2015). Variance of light wavelength and intensity from reflected surfaces can affect animal behavior with regard to interactions between stimuli received by photoreceptors (Kelber et al. 2003). This phenomenon has been observed in aphids (Doering and Chittka 2007), specifically color response by *Myzus persicae* Sulzer and *Aphis fabae* (Scopoli) was based on positive stimuli received from the green spectrum and negative stimuli from the blue/UV spectrum (Moericke 1950, 1955, 1979). Similar behavior has also been observed by the flies *Dacus oleae* (Tephritidae) and *Eristalis tenax* (Syrphidae) for the purposes of oviposition site selection and feeding, respectively (Prokopy et al. 1975, Lunau and Wacht 1994, Kelber 2001). Although antagonistic interactions between Hessian fly photoreceptors have not been studied, Harris et al. (1993) did demonstrate that reflected wavelengths in the region of 400 – 500 nm inhibited female Hessian fly landing. Therefore, Hessian fly may have blue- and green-sensitive photoreceptors that interact, and the interaction between those photoreceptors could affect the fly's response to colors. The broad spectra of light reflected from targets and photoreceptor interactions may explain why Hessian flies preferred the yellow-green targets more than the green targets in the study by Harris et al. (1993). The LEDs used in our experiment emitted light in a relatively narrow range (± 10 nm), which avoided potential interactions between blue- and green-photoreceptors. Without those photoreceptor

interactions affecting their response to LEDs, the Hessian flies in our experiment responded to specific narrow wavelength ranges in the lower range of the green spectrum (502 nm and 525 nm) more than the hypothesized yellow-green LED (565 nm) based on the results of Harris et al. (1993).

Another noteworthy result from our bioassays is the response difference to LEDs by each sex in the final bioassay, 90% female response and 51% male response (Fig 3.3C). The lower response by males is not surprising, as the primary interest of adult males is to locate females for mating, and males locate females using the female sex pheromone, not light (Harris and Foster 1991). Higher response by females to LEDs was foreseen as Harris and Rose (1990) demonstrated female Hessian fly response to visual stimuli for oviposition site selection. The interesting component of female attraction to LEDs is the importance of light wavelength and intensity, as female response to LEDs changed considerably from the first bioassay (55%) to the final bioassay (90%) after LED wavelength and intensity had been fine-tuned.

Tailoring wavelength and intensity to a specific species to increase attraction of that species has been observed in several other insects (Wilton and Fay 1972, Bishop et al. 2004a, Bishop et al. 2004b, Snyder et al. 2016). Knowledge of a species response to different wavelengths and intensities is imperative to understanding insect response to visual targets. Our study demonstrates both Hessian fly sexes are attracted to visual stimuli, females significantly more than males, and each sex has different sensitivity to LED wavelength and intensity, with females attracted to a narrower wavelength range than males. Attraction by both Hessian fly sexes to LEDs in these bioassays indicates that LEDs could be added to Hessian fly pheromone traps to increase male and female capture; however, further experiments are needed to validate this hypothesis.

While these initial findings are important, a better understanding of Hessian fly behavioral response to emitted light sources is needed before LEDs can be incorporated into existing Hessian fly traps. Future works examining potential interactions between environmental factors and Hessian fly response to LEDs is critical. One environmental factor that may interact with Hessian fly response to LEDs is the sun, which could mitigate the intensity of the LEDs as male Hessian flies are active from 0100 to 1100 and females demonstrate oviposition activity from 0900 to 1300 (Harris and Rose 1989, Bergh et al. 1990). Additionally, due to the small size of the light arena (25 cm × 25 cm), Hessian fly response to LEDs at various spatial scales needs to be assessed. Interactions between LEDs and female produced sex-pheromone or the sex-pheromone lure also needs to be studied, and how Hessian flies integrate the chemical pheromone cue with the visual cue of LEDs deserves further investigation. All of these interactions and more will lead to a better understanding of Hessian fly attraction to LEDs, which can be incorporated into traps and aid in monitoring strategies for both sexes of Hessian fly.

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Tables and Figures

Figure 3.1. Schematic of the bioassay light arena (view from above) used to test Hessian fly attraction to LEDs at varied wavelengths and intensities.

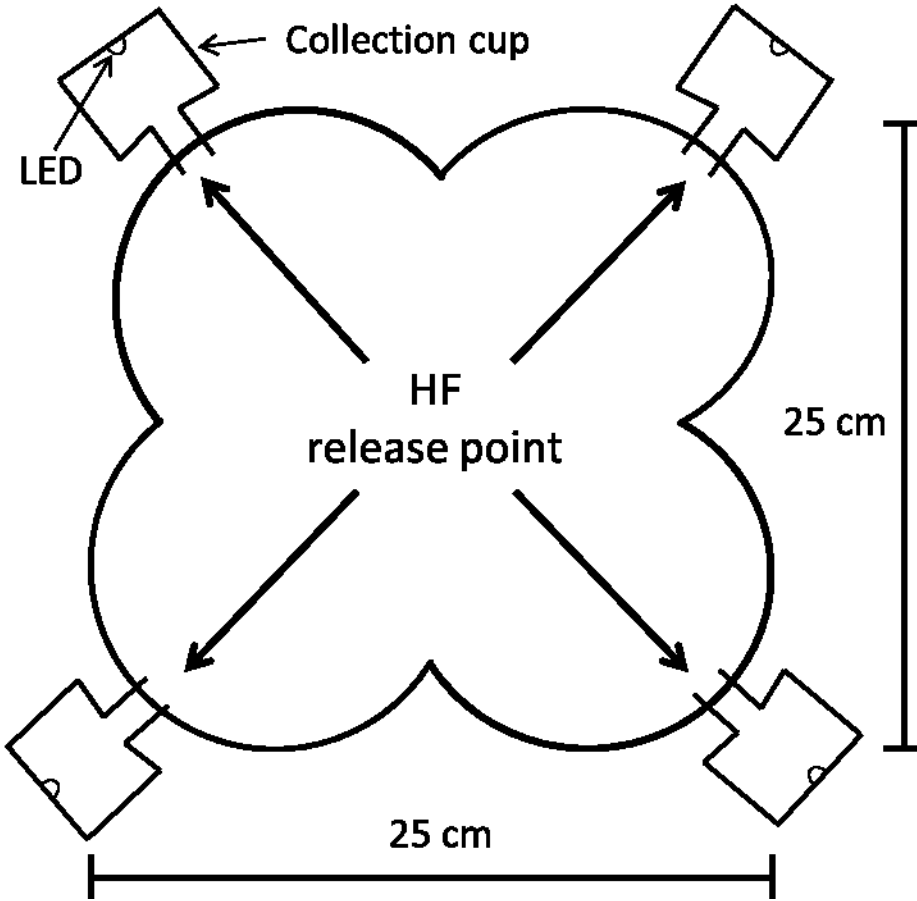


Figure 3.2. Mean percentage (\pm standard error of the mean or SEM) of female (A-C) and male (D-F) Hessian fly choice of A, D) LED color (blue = 472 nm, green = 527 nm, amber = 590 nm, and red = 624 nm); B, E) wavelengths with the green spectrum (502 nm; 525 nm; and 565 nm) and white; and C, F) intensity (4 W/m², 8 W/m², 12 W/m², and 16 W/m²). Bars with the same letter above are not statistically different ($\alpha = 0.05$).

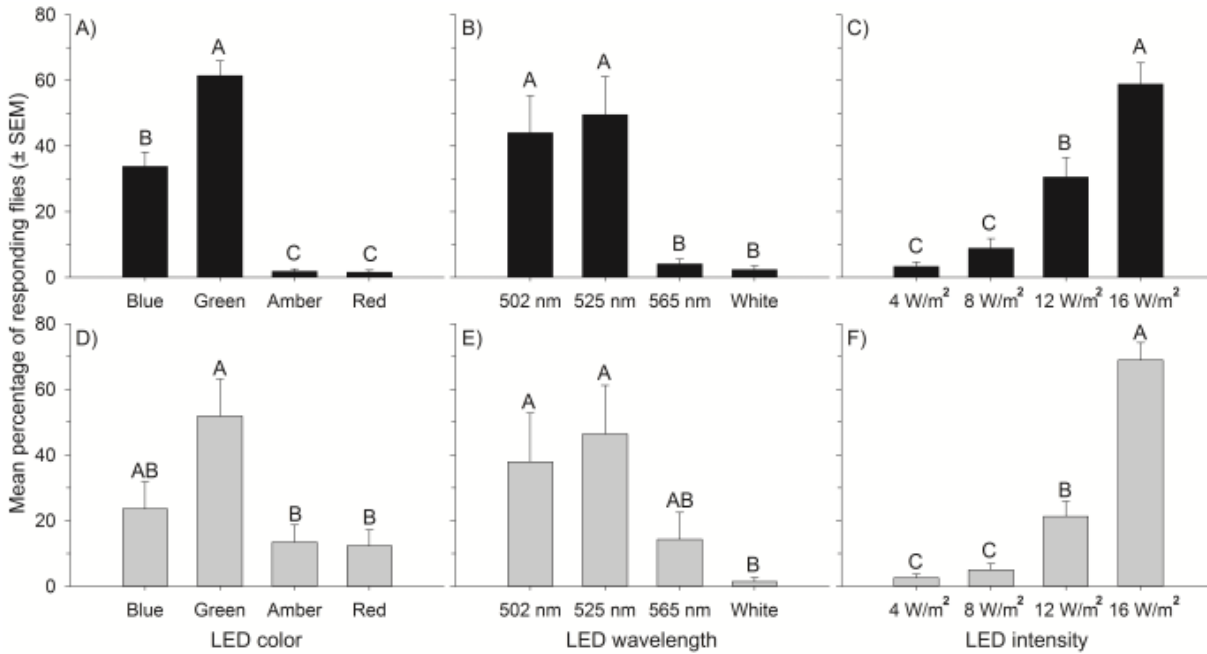
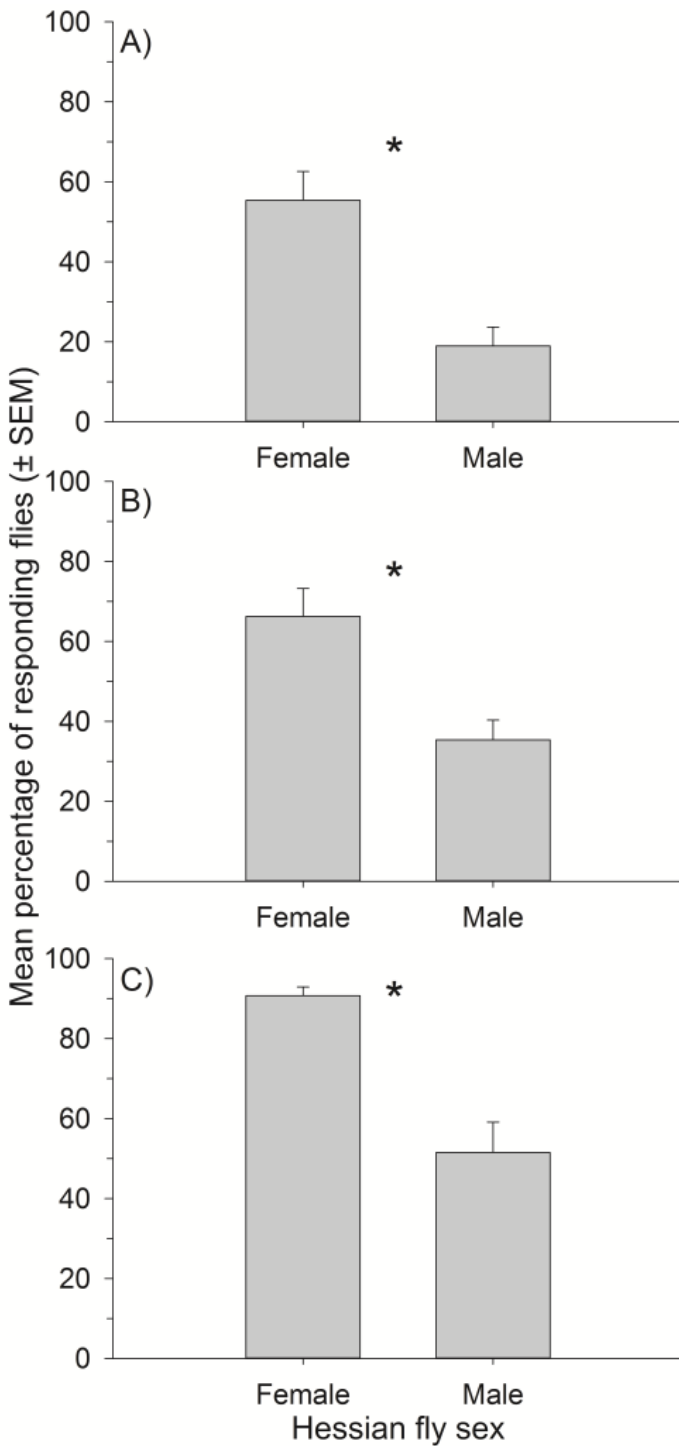


Figure 3.3. Mean percentage (\pm standard error of the mean or SEM) of each Hessian fly sex attraction to all LEDs in A) broad-spectrum bioassay (n = 8 reps), B) green-spectrum bioassay (Female: n = 7 reps, Male: n = 8 reps), and C) light intensity bioassay (Female: n = 8 reps, Male: n = 7 reps). Asterisk denotes a significant difference between male and female attraction to LEDs at $\alpha = 0.05$.



Chapter 4 - Response of Hessian Fly, *Mayetiola destructor* (Diptera: Cecidomyiidae), to Combinations of Light Emitting Diodes and Female Sex Pheromone

Introduction

The Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), is a major wheat (*Triticum* spp. L.) pest with a global distribution (CABI 2016), capable of causing up to 386 kg/ha (5.74 bu/ac) yield loss for each Hessian fly immature per tiller in some production systems (Buntin 1999; Smiley et al. 2004; Alvey 2009). Yield loss is caused by larval feeding; resulting in stunted growth and eventual death of seedlings, lodging, or reduced seed heads of mature plants (Buntin 1999; Harris et al. 2003; Whitworth et al. 2009; Schwarting et al. 2016). Several management practices have been developed to mitigate the severity of damage caused by Hessian fly. These practices include destruction of volunteer wheat before planting, increased use of resistant cultivars, adherence to planting dates that escape early fall infestations (i.e., fly-free dates), and insecticidal seed treatments (Schmid et al. *In review*). Historically, these management practices are implemented on a prophylactic basis, typically owing to historical crop failures for a given production field. Prophylactic implementation of Hessian fly management is problematic because while Hessian fly infestations can cause significant yield reduction annually across regions (Buntin 1999), major infestations are often localized (Hatchett et al. 1981; Alvey 2009). The decision to implement IPM against this often localized, but potentially significant pest, must be balanced against other important production factors. For example, Hessian fly resistant cultivar versus a higher yielding cultivar, planting after fly-free dates versus poor forecasted weather conditions for planting, or application of seed treatments versus wheat commodity prices. In an effort to inform the decision-making process for producers

and prevent implementation of unwarranted management practices, a monitoring method for this pest has been extensively studied (Anderson et al. 2012; Schwarting et al. 2015; Knutson et al. 2017).

The current Hessian fly monitoring strategy consists of a synthetic pheromone-baited sticky trap, which attracts male Hessian flies with a synthetic Hessian fly female sex pheromone lure identified by Andersson et al. (2009). Deployment of the pheromone trap has proven to be a reliable detection method, detecting low densities of male Hessian flies under field conditions (Anderson et al. 2012; Schwarting et al. 2015; Knutson et al. 2017). Producers can use trap data to learn occurrence and level of adult activity in localized areas prior to and after planting (Anderson et al. 2012; Bradford 2014; Schwarting et al. 2015; Knutson et al. 2017). For example, early detection of brood emergence prior to planting can aid planting date decisions, as weather conditions can cause brood emergence to vary from one year to the next (Drake and Decker 1932; Byers and Gallun 1972). Additionally, trap data can inform producers of supplementary brood occurrence throughout the growing season, warning producers to check for Hessian fly infestations and limit crop inputs or switch fields from grain production to livestock forage if needed (Knutson et al. 2017). Despite the benefits associated with monitoring for male Hessian flies using pheromone-based traps, male trap captures are not shown to correlate with Hessian fly infestation in the surrounding field; where positive captures do not consistently reflect an economically-significant infestation level (Schwarting et al. 2015; Knutson et al. 2017). Although the benefit of monitoring for female Hessian flies has not been studied, female Hessian flies are the primary agent of dispersal through selection of oviposition sites (Harris and Rose 1989). Moreover, monitoring for females of other pest fly species has been valuable to improving their management (Bowden and Jones 1979; Hendrichs 1999; Broughton and Rahman

2017). Therefore, incorporation of a female monitoring method into the current Hessian fly pheromone trap may improve predictions between trap capture and field infestation, leading to the justified implementation of Hessian fly management practices. Thus, the development of a method to monitor for females will be needed.

Several cues from the environment affect female Hessian fly dispersal during oviposition site selection. Wind speed and direction, spatial configuration of plants, composition of host and non-host plants in an area, and specific characteristics of host plants all affect female Hessian fly movement within the environment (Harris and Rose 1990; Withers and Harris 1996; Withers and Harris 1997; Withers et al. 1997). Specific plant characteristics utilized by females to determine host suitability include chemical, tactile, and visual cues (Harris and Rose 1990). Although all of these plant characteristics are important for oviposition, visual cues consisting of yellow-green light, vertical contours, and a larger overall area are most important for orientation to and landing on host plants by female Hessian flies (Harris et al. 1993). Also, recent research has shown that both female and male Hessian flies are attracted to green light emitting diodes (LEDs) set to specific wavelengths (525 nm) at high intensities (16 W/m²) (Schmid et al. 2017). Incorporation of these LEDs into the current Hessian fly pheromone trap is a potential solution for estimating the female portion of the Hessian fly population in a production field.

Knowing how both sexes of Hessian fly respond to multiple attractants could impact Hessian fly management beyond trap design. This information could lead to better understanding of Hessian fly dispersal, which would be valuable for predicting vulnerable fields or areas within a field prior to planting, and consequently implementing prophylactic Hessian fly IPM practices. However, further research on Hessian fly behavioral response to a combination of the attractants is needed. As observed with other insects, the addition of known olfactory attractants does not

always elicit the expected behavioral response (Schroeder and Hilker 2008; Bruce and Pickett 2011; Riffell 2012). For example, when a known repellent (CO₂) of *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) was combined with an attractant (apple cider vinegar), the addition of the repellent to the attractant actually increased fly attraction (Faucher et al. 2013). Additionally, several insects will actually avoid pheromones for many reasons, such as intraspecific competition, inbreeding during mating, and competition for oviposition sites (Prokopy et al. 1976; Byers 1993; Herzner et al. 2006). Therefore, it is plausible that female Hessian flies could avoid the sex pheromone lure as a means to avoid intraspecific competition for mates and oviposition sites. However, during oviposition site selection, female movement during flight is first directed toward visual stimuli and subsequently followed by olfactory and tactile stimuli once the female is within 1 cm of the potential oviposition site (Harris and Rose 1990; Harris et al. 1993). Owing to the integration of visual, chemical, and tactile cues by female Hessian flies, with the importance of visual cues to first direct flight, our hypothesis was that the sex pheromone lure will not decrease female Hessian fly attraction to green LEDs. Also, we hypothesized green LEDs would increase male Hessian fly attraction to the sex pheromone lure, as males are attracted to both stimuli (Andersson et al. 2009; Schmid et al. 2017). Therefore, the objective of this experiment was to examine the response of male and female Hessian flies to each attractant individually, and in combination.

Materials and Methods

Insect Rearing. Hessian flies used in this study were from a laboratory reared colony, originally collected in 2005 from wheat fields in Scott County, KS (Chen et al. 2009a). All flies in the colony were of the biotype Scott-KS-GH-05 (Chen et al. 2009b). The colony was reared on susceptible wheat seedlings (var. Karl 92) in a greenhouse over a 29 – 35 d generation period

(Tan et al. 2013), at 22°C, and a 16:8 h (light:dark) photoperiod. Flies used in the study were all newly eclosed (<12 h) the previous night or the morning of each bioassay replicate.

Y-tube bioassays. Binary-choice bioassays were conducted in Y-tube olfactometers (Fig. 4.1) to assess attraction of male and female Hessian fly to green LEDs (525 nm, 16 W/m²) and synthetic female Hessian fly sex pheromone lure (Pheronet AB; Alnarp, Sweden). The Y-tubes were constructed from polyvinyl chloride (PVC) tubes. The inner diameters of the Y-tubes were 3.5 cm, with the length of arms and stem measuring 26 and 33 cm respectively. Windows were cut into the upper portion of each arm (2.1 × 3.5 cm) and the stem (10 × 3.5 cm). Each window was sealed with clear plastic sheeting. Air was pulled through the Y-tube from the stem, flowing first through charcoal filters at the end of each arm. Airflow averaged 1.8 m/s, but airflow varied slightly during each 23 h bioassay due to fluctuations in air compressor flow.

LED bulbs (5-mm round through-hole bulb, Part # C503B-GCS-CY0C0791, Cree Inc.; Durham, NC) were positioned in the center of the tube at the distal end of the Y-tube arm. To test the Hessian fly female sex pheromone, the synthetic lure was placed in an air tight plastic bag with a piece of filter paper (GE Healthcare Bio-Sciences; Pittsburgh, PA) for at least 24 h prior to each bioassay replicate. Strips of filter paper (5.5 × 1.5 cm) were removed from the plastic bag immediately prior to the start of each replicate. Filter paper strips were folded in the center to produce an A-frame shape and positioned in the center of the tube bottom at the distal end of the Y-tube arm.

Male and female Hessian flies were tested in separate Y-tubes. During testing, newly eclosed Hessian flies were removed from lab colony at 0700 h and transferred to a chamber located on the distal end of the Y-tube stem at 0730 h for the start of each bioassay replicate. Flies remained in the chamber for 1 h to let individuals acclimate to the Y-tube environment. The

number of individuals used per replicate varied between 9 – 11 flies due to difficulty transferring live flies into the Y-tube chamber. The variance in number of individuals used per replicate was accounted for during data analysis. After 1 h, flies were released from the chamber, and the appropriate attractants (LEDs and pheromone) were placed in the distal ends of the Y-tube arms. Flies remained in the Y-tube for 23 h to allow individuals sufficient time to make a choice. A response was defined as movement by an individual fly a minimum of 10.3 cm up one of the arms. To prevent flies from moving to other areas of the Y-tube after making a choice, each arm of the Y-tube contained a mesh funnel that was 10.3 cm up the arm with Tangle-Trap Sticky Coating (The Tanglefoot Company; Grand Rapids, Michigan) covering the interior of the tube on the distal side of the funnel.

Four control bioassays ($n = 3$ replicates per bioassay) were performed to test olfactory arena construction and attractants for potential biases. Control bioassays included the following arrangements in the Y-tube arms: green LED versus blank (nothing placed in the arm), pheromone versus blank, blank versus blank, and green LED versus green LED. Control bioassays green LED versus blank and pheromone versus blank were run before the treatment bioassays to ensure flies were responding to each attractant in the Y-tube. Since PVC used to construct Y-tubes is a plastic and volatiles from the pheromone may adhere to plastics, potentially biasing the results of the treatment bioassays, we ran control bioassays (blank versus blank and green LED versus green LED) after treatment bioassays to ensure that volatile levels were maintained. The green LED versus green LED (a cue that attracted both males and females) bioassay was performed to demonstrate that any pheromone that potentially adhered to the pheromone arm of the Y-tube did not bias choice toward the arm during the treatment bioassays. Similarly, the blank versus blank bioassay was performed to confirm that pheromone that

potentially remained in the Y-tube did not bias movement of flies down the arm of the Y-tube during the treatment bioassays. Treatment bioassays (n = 8 replicates per bioassay) were performed after completion of the control bioassays green LED versus blank and pheromone versus blank. The three treatment bioassays were: 1) green LED versus pheromone, 2) green LED + pheromone versus green LED, and 3) green LED + pheromone versus pheromone; hereafter referred to as bioassays 1, 2, and 3, respectively.

Data Analysis. To account for unequal sample sizes, proportion Hessian fly response was used to assess fly attraction to the LED and pheromone treatments; however, by proportioning the data of Hessian fly response, the distribution of results was restricted between 0 and 1. Consequently, some of the bioassays failed the normality distribution assumption for analysis of variance (ANOVA). Bioassays that failed the Shapiro Wilk test for normality included female treatment bioassays 1 and 3; both male and female control bioassays blank versus blank, and LED versus blank; and female control bioassay pheromone versus blank. Proportion Hessian fly response to an individual Y-tube arm for each replicate was calculated by dividing the number of responsive flies collected from each arm by the total number of Hessian flies released into the Y-tube for that replicate. Proportion male and female Hessian fly response data were subjected to a one-way ANOVA (R64 version 3.0.1, Boston, MA) for the main effect of attractant in each bioassay. Means were separated using the Tukey-Kramer option to test for statistical significance between the attractants at a significance level of $\alpha = 0.05$.

Results

Control Bioassays. Four control bioassays (Fig. 4.2) were performed to assess male and female attraction to the green LED and pheromone, and also test for potential biases due to adherence of pheromone Y-tube arena. Table 4.1 shows an overall significant difference between

choice options for every control bioassay, except the green LED versus green LED bioassay for males. However, neither male nor female Hessian flies demonstrated significant preference for the choice options in either arm (green LED and green LED) of the Y-tube bioassay (Figs. 4.2C, D). The results of this bioassay revealed the flies did not favor one LED bulb more than the other and did not favor one arm of the arena more than the other. The blank versus blank bioassay showed that neither male nor female Hessian fly moved far enough down either Y-tube arm to be counted as a choice when no attractant was present (Figs. 4.2A, B). Together, the results of these two control bioassays demonstrated any pheromone that adhered to the PVC during the treatment bioassays did not attract Hessian fly to move from the stem to the arms, and did not affect fly choice of either arm. Two additional control bioassays were performed prior to the treatment bioassays to assess Hessian fly response to the attractants within the Y-tube arena. In the green LED versus blank bioassay, both males and females chose the green LED significantly more than the blank arm (Fig. 4.2E, F); however, in the pheromone versus blank bioassay only males were attracted to the pheromone (Fig. 4.2G), but females were not (Fig. 4.2H). These bioassays confirm the expected responses of each Hessian fly sex to the attractants, with males attracted to both the green LED and pheromone, and females attracted to only the green LED.

Treatment Bioassays. The green LED significantly attracted male Hessian fly, whether in combination with the pheromone or standalone, in all bioassays (Fig. 4.3A, C, E; Table 4.1). This was demonstrated when males chose the LED arm 41% more than the pheromone arm in bioassay 1 (Fig. 4.3A), and again in bioassay 3 when males chose the LED + pheromone arm 51% more than the pheromone arm (Fig. 4.3E). In bioassay 2, the combination of the pheromone with the green LED did result in male choice of the green LED and pheromone combination significantly more, 35%, than the standalone green LED (Fig. 4.3C).

Female Hessian fly choice in all bioassays was motivated by the green LED and not the pheromone (Fig. 4.3B, D, F). In a direct comparison of the two attractants in bioassay 1, females chose the LED arm 81% more than the pheromone arm (Fig. 4.3B). Also, in bioassay 3, the arm containing the green LED and pheromone had a significantly higher response, 70%, from females than the arm containing only the pheromone (Fig. 4.3F). The inability of the pheromone to attract females was highlighted in bioassay 2, where no significant difference was observed between the two arms (Fig. 4.3D). Another important result from bioassay 2 was the pheromone lure did not deter female Hessian flies from the green LED and most female made a choice during the bioassay, i.e., only a small proportion of females remained in the starting portion of the tube.

Discussion

The results of these bioassays demonstrate green LEDs (525 nm, 16 W/m²) elicit a greater attraction by both Hessian fly sexes than the synthetic Hessian fly female sex pheromone lure, and perhaps more importantly, neither attractant acted as an antagonist to deter Hessian fly response to the other attractant. Control bioassays showed each sex is attracted to the green LED, a result consistent with our previous research (Schmid et al. 2017), and only male flies were attracted to the pheromone, which was expected. Treatment bioassays showed both sexes of Hessian fly respond to the green LED in the presence of the female sex pheromone. However, a striking result from treatment bioassay 1 was male preference for the green LED over the sex pheromone when given a choice (Fig. 4.3A). However, the sex pheromone did increase male choice of the green LED, demonstrated in treatment bioassay 2 (Fig. 4.3C). Equally important as the male results, were female responses to the green LED and pheromone. These bioassays demonstrate female Hessian flies will respond positively to the green LED in the presence of the

sex pheromone (Fig. 4.3F), and also the pheromone does not deter female Hessian fly behavioral response to the LED (Fig. 4.3D). These bioassays show green LEDs and the synthetic Hessian fly female sex pheromone can be used in combination without hindering Hessian fly behavioral response to either attractant.

A surprising result from our bioassays was male Hessian fly preference of the green LED over the sex pheromone. Previous research demonstrated male Hessian flies use the female sex-pheromone to guide their movement (McKay and Hatchett 1984; Harris and Foster 1991). Our hypothesis, based on this research, was males would be attracted to the pheromone more than the LED, but the LED would increase male attraction to the pheromone when the two stimuli were combined. While it was unexpected males preferred the green LED over the sex pheromone, it is not unreasonable. Many insects demonstrate taxis to a combination of visual and olfactory cues when searching for food, mates, oviposition sites, and refugia (Bell 1990; Rojas and Wyatt 1999; Jang et al. 2000; Bjorklund et al. 2005). Since searching behavior is essential to the acquisition of resources needed for growth, development, and success of future generations, utilizing multiple sensory stimuli helps organisms to respond efficiently to variation in complex environments (Gegeer 2005; Hebets and Papaj 2005). Even female Hessian flies display this behavior using a combination of olfactory, visual, and tactile cues for oviposition site selection (Harris and Rose 1990). Male Hessian flies may be using multiple stimuli to more efficiently search for resources, similar to the way female Hessian flies use multiple environmental cues for oviposition site selection.

However, it is assumed male Hessian flies have no need for oviposition site selection behavior, as most of its adult life is spent either searching for mates or mating (Bergh et al. 1990). After emergence, Hessian fly adults mate within a few hours (Bergh et al. 1990). It is

known that males use the pheromone released by females to locate potential mates close to the site of emergence (McKay and Hatchett 1984; Bergh et al. 1990). Consequently, if males are strongly dependent on use of pheromones to locate mates; then the question becomes, why are males attracted to green LEDs? The answer could be that males use a hierarchy in responses, similar to female Hessian fly response to cues during oviposition. Color visual cues could be used first to indicate that they are in an environment that females are likely to be located and then they tend to be more responsive to the pheromone. Males may also use visual cues in addition to pheromone cues to give them a competitive advantage over other males to find mates.

Integrating a visual cue (i.e., green LED or green light) with the female sex pheromone may give male Hessian flies an additional search tactic to find mates. Males of other insect species have been shown to use multiple search tactics to locate mates (Bell 1990). For example, male wood butterflies, *Pararge aegeria*, switch between two tactics to locate females, perching and patrolling, depending on the availability of sunspots (Davies 1978). The searching tactic used can be dictated by the environment (Bell 1990). It is known that wind affects Hessian fly dispersal, and males have been caught in screens 183 m downwind from Hessian fly infested fields (McColloch 1923; Withers and Harris 1997). Therefore it is plausible that males use visual cues when caught in wind gusts, in addition or in combination with pheromone cues, to aid in the location of mates or host plant habitat, much the way aphids use visual cues related to host plant wavelengths to determine when to land during migratory flights (Parry 2013). Further investigation is needed to determine how and why male Hessian flies use visual cues as adults. Regardless, our results show that green LEDs increase attraction of males to the female sex pheromone lure.

While our results show significant Hessian fly attraction to green LEDs in the presence of the sex pheromone lure, differences in environmental conditions between the Y-tube arena and commercial wheat fields may affect Hessian fly attraction to green LEDs under field conditions. Ambient light, or sunlight, is a potential factor hindering Hessian fly attraction to LEDs under field conditions, as solar radiation (sunlight) varies throughout the day and with cloud cover (Garg and Prakash 2000; Burgess 2009). For example, intensity and direction of ambient light has been shown to affect the attractiveness of *Rhagoletis indifferens* Curran (Diptera: Tephritidae) to different visual traps (Yee 2015). It is important to note that Hessian fly attraction to green LEDs both in this study and previous work by Schmid et al. (2017) was conducted in the absence of ambient light. Consequently, it is not known if ambient light will affect Hessian fly attraction to LEDs, as was observed with *R. indifferens*. Another major difference between a commercial wheat field and our experimental arenas is size. The Y-tube arena used in this study was only 59 cm in length, a relatively short distance compared to the size of a typical wheat field. Previous research also used small arenas, 25 × 25 cm, to assess Hessian fly attraction to LEDs (Schmid et al. 2017). Granted the response scale of Hessian flies to LEDs does not need to be the area of an entire field; however, determining the attraction radius of an LED would be critical to understanding Hessian fly response under field conditions. The potential for variations in ambient light and size, or other environmental factors, of commercial wheat fields to affect Hessian fly response to LEDs is plausible, and these concerns warrant further investigation before it is known that green LEDs will improve male Hessian fly trap capture in commercial wheat fields.

These bioassays demonstrate that green LEDs will not hinder the attraction of either Hessian fly sex to the synthetic Hessian fly female sex pheromone lure, and green LEDs may

increase male attraction to the pheromone. Traps that attract both female and male Hessian flies have the potential to better correlate trap captures with field infestation, as females are responsible for oviposition site selection. The power to monitor Hessian fly presence in an area with a cheap and effective trap will allow wheat producers needed time to implement management strategies (i.e., fly-free dates, destruction of volunteer wheat before planting, and insecticidal seed treatments) while also helping to improve monitoring for potential Hessian fly invasions to areas with no previous Hessian fly presence.

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Tables and Figures

Table 4.1. ANOVA results for Y-tube bioassays examining male and female Hessian fly attraction to combinations of green LED (525 nm, 16W/m²) and Hessian fly female sex pheromone lure.

Bioassay	df	F	P-value
<u>Males</u>			
Blank vs. Blank	2, 6	4.85e ³¹	≤0.01
Green LED vs. Green LED	2, 6	1.50	0.30
Green LED vs. Blank	2, 6	78.00	≤0.01
Pheromone vs. Blank	2, 6	38.48	≤0.01
Pheromone vs. Green LED	2, 21	9.03	≤0.01
Pheromone + Green LED vs. Green LED	2, 21	13.89	≤0.01
Pheromone + LED vs. Pheromone	2, 21	42.13	≤0.01
<u>Females</u>			
Blank vs. Blank	2, 6	4.85e ³¹	≤0.01
Green LED vs. Green LED	2, 6	10.05	0.01
Green LED vs. Blank	2, 6	366.00	≤0.01
Pheromone vs. Blank	2, 6	406.50	≤0.01
Pheromone vs. Green LED	2, 21	107.10	≤0.01
Pheromone + Green LED vs. Green LED	2, 21	9.53	≤0.01
Pheromone + LED vs. Pheromone	2, 21	21.10	≤0.01

Figure 4.1. Photo of the Y-tube arena (view from above) used to test Hessian fly attraction to green LEDs and the synthetic female Hessian fly sex pheromone.

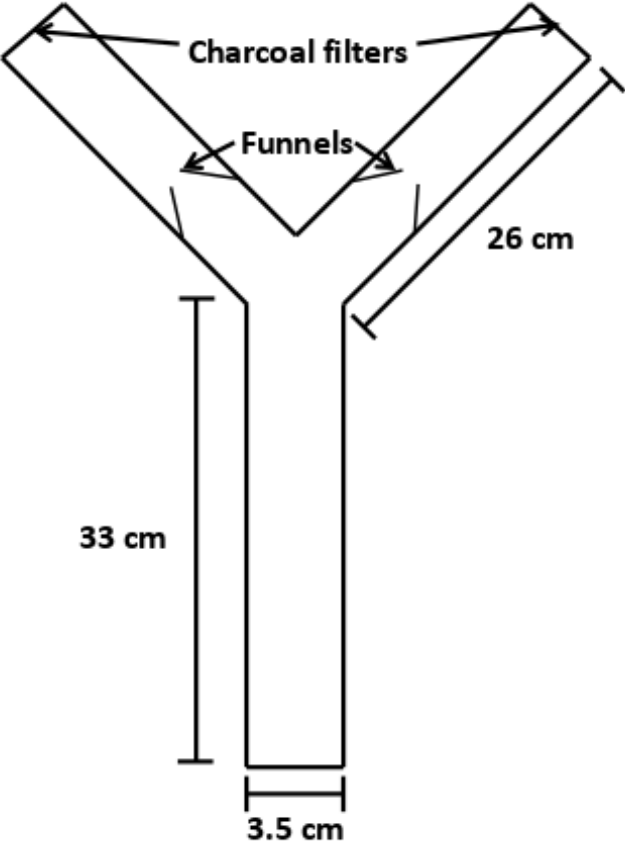


Figure 4.2. Mean proportion (+ standard error of the mean or SEM) of male (A, C, E, G) and female (B, D, F, H) Hessian fly choice within Y-tube arena between A, B) blank arm and blank arm; C, D) green LED arm (Gr LED) and green LED arm (525 nm, 16 W/m²); E, F) green LED arm and blank arm; and g, h synthetic female Hessian fly sex pheromone arm (Pher) (Pheronet AB, Alnarp, Sweden) and blank arm. Bars with the same letter above are not statistically different ($\alpha = 0.05$).

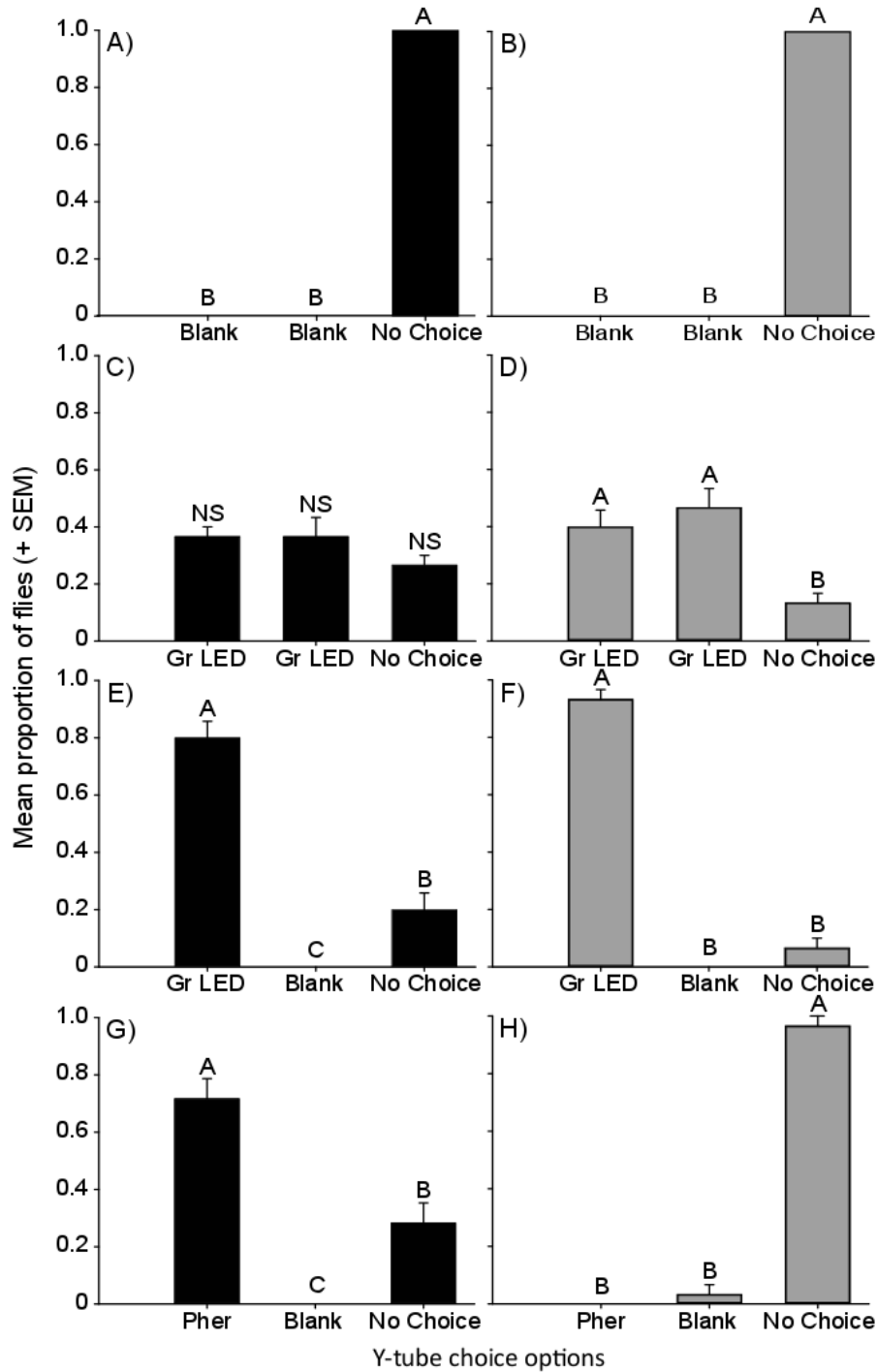
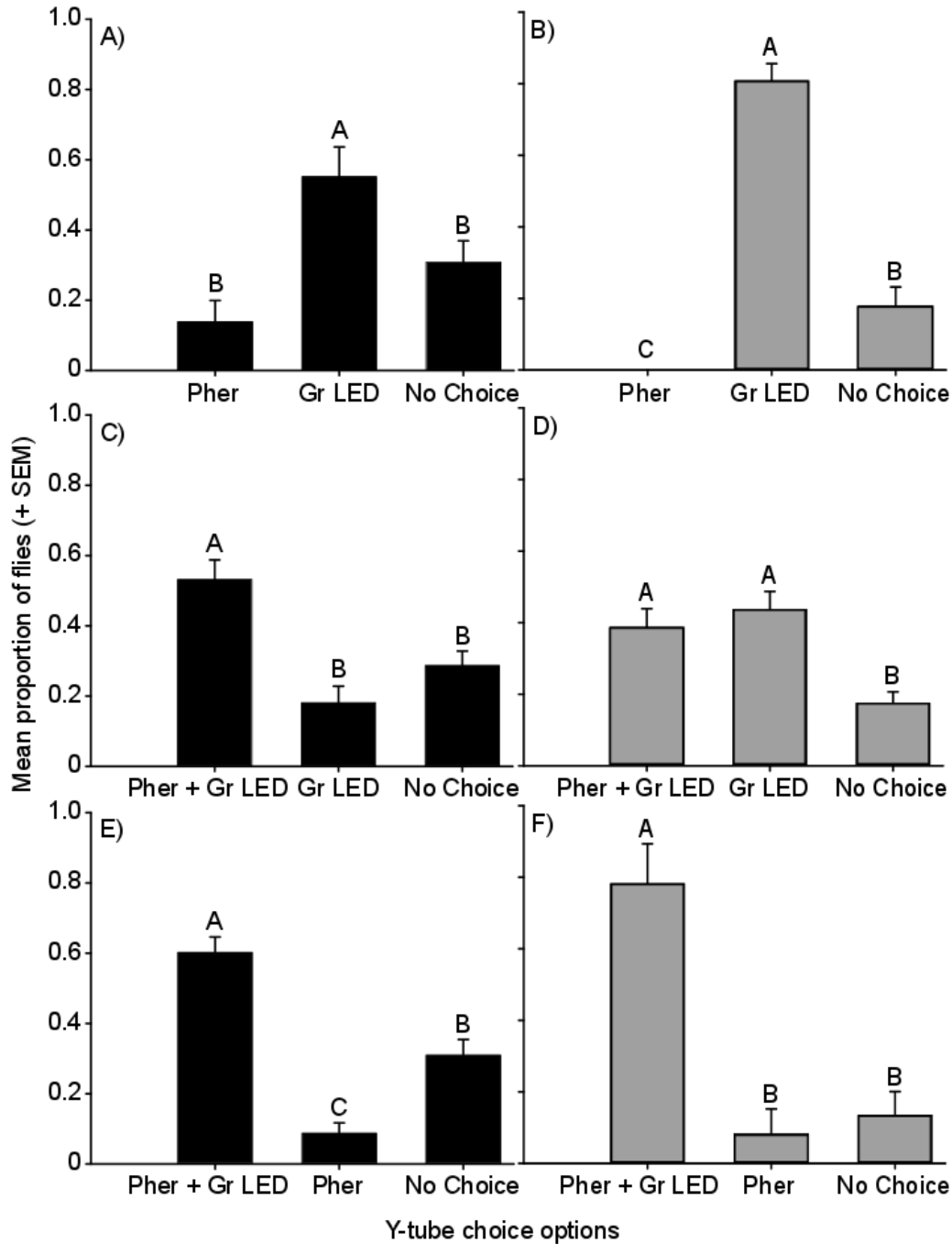


Figure 4.3. Mean proportion (+ standard error of the mean or SEM) of male (A, C, E) and female (B, D, F) Hessian fly choice within Y-tube arena between A, B) synthetic female Hessian fly sex pheromone arm (Pher) (Pheronet AB, Alnarp, Sweden) and green LED arm (Gr LED) (525 nm, 16 W/m²); C, D) green LED with synthetic female Hessian fly sex pheromone arm and green LED arm; and E, F) green LED with synthetic female Hessian fly sex pheromone arm and synthetic female Hessian fly sex pheromone arm. Bars with the same letter above are not statistically different ($\alpha = 0.05$).



Chapter 5 - Hessian Fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), Attraction to Light Emitting Diodes Under Laboratory and Field Conditions

Introduction

The Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), is historically regarded as one of the principal pests of wheat in the United States (Webster and Kelly 1915, Walton 1920, Barnes 1956). Despite the significance of this pest, economic damage is often sporadic and limited to local events (Harris and Foster 1999, Alvey 2009). The sporadic distribution of field infestations is problematic to the implementation of prophylactic Hessian fly integrated pest management (IPM) methods. Specific methods include planting resistant cultivars, adherence to planting dates that escape early fall infestations (fly-free dates), use of insecticidal seed treatments, and destruction of volunteer wheat before planting (Schmid et al. *In review*). Without a pre-planting monitoring strategy for adult Hessian fly activity, wheat farmers will be less likely to adopt preventive strategies to manage this pest, since information on pest severity is lacking at critical times.

Traps utilizing synthetic lures of the Hessian fly female sex pheromone, identified by Harris Foster et al. (1991) and Andersson et al. (2009), are the currently developed strategy to monitor male Hessian fly populations (Anderson et al. 2012, Schwarting et al. 2015, Knutson et al. 2017). However, high capture rates of males do not correlate with infestations in the surrounding field (Schwarting et al. 2015, Knutson et al. 2017), and these traps have not been widely adopted by farmers to monitor for adult male activity. Thus, Hessian fly monitoring methods need further refinement to accurately predict field infestations, which includes quantifying the contributions made to population dynamics by females. Therefore, developing

effective methods to monitor female Hessian fly adults is foundational to correlating adult activity with infestation levels in the field, as females are the primary agent of dispersal through oviposition site selection (Harris and Rose 1989). Although direct correlations between female Hessian fly activity and field infestations has not been studied, monitoring female activity for other fly species has improved control of those pests (Bowden and Jones 1979, Hendrichs 1999, Broughton and Rahman 2017). For example, trap captures of the wheat bulb fly, *Delia coarctata* (Fallen) (Diptera: Anthomyiidae), correlate with the number of eggs per hectare, and this information can be used to advise the use of seed treatments for winter wheat (Bowden and Jones 1979). In our study system, monitoring female activity may prove useful for making field-based management decisions. A critical first step to understanding the utility of such practices depends on reliability in trapping female flies.

Light emitting diodes (LEDs) are an affordable, efficient attractant used to monitor for several insect species including multiple *Culicoides* spp. (Diptera: Ceratopogonidae), *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), and *Euscepes postfasciatur* (Fairmaire) (Coleoptera: Curculionidae) (Chu et al. 2003, Bishop et al. 2004, Nakamoto and Kuba 2004, Snyder et al. 2016). In the laboratory, both sexes of Hessian fly are attracted to green LEDs set to a specific wavelength and intensity (525 nm, 16 W/m²), though females elicit a stronger response (1.8 times higher) compared to males (Schmid et al. 2017). Furthermore, both sexes are attracted to green LEDs in the presence of the synthetic female Hessian fly sex pheromone lure, which indicates the potential to incorporate green LEDs into existing Hessian fly pheromone trap designs, providing a mechanism to monitor males and females simultaneously (Schmid et al. *In review*).

Before LEDs are fully incorporated into existing Hessian fly trap designs, it is imperative to consider environmental factors that can affect Hessian fly behavior and response to LEDs under field conditions. Ambient light, predominately provided by sunlight, can potentially influence the level of insect attraction to LEDs incorporated into traps. For example, ambient light intensity and direction has been shown to affect the attraction of *Rhagoletis indifferens* Curran (Diptera: Tephritidae) to visual traps (Yee 2015). Similarly the variation in solar radiation within a day (Garg and Prakash 2000, Burgess 2009) could affect adult Hessian fly attraction to LEDs, owing to adult activity primarily beginning a few hours before sunrise and continuing until early morning for mating and oviposition purposes (Bergh et al. 1990, Withers and Harris 1996). Therefore, it is plausible that ambient light will affect Hessian fly attraction to LEDs after sunrise. Wheat odor within the trapping environment is another potential competing stimuli affecting Hessian fly attraction to LEDs, since female Hessian flies use wheat-derived odors to determine suitable oviposition sites (Foster and Harris 1992, Harris et al. 1993). Although females use wheat odors to select oviposition sites, it is not known whether male Hessian flies respond to wheat odor. If LED traps are going to be developed as a tool to monitor for Hessian fly, it is important to better understand how males interact with this new visual stimuli in the presence of other common environmental cues, and this knowledge gap should be addressed before LEDs are incorporated into traps deployed in the field.

The objective of this study was to examine the effect of ambient light and wheat odor on Hessian fly attraction to LEDs first in the laboratory, and then under field conditions. We predicted that ambient light would decrease both sexes attraction to LEDs. Also, because female Hessian flies use wheat odor to help locate oviposition sites, we predicted that combining wheat odor with a green LED would increase female attraction. However, male attraction to a green

LED would be unaffected by wheat odor, as males have only shown attraction to the sex pheromone and green LEDs (Harris and Foster 1991, Schmid et al. 2017).

Materials and Methods

Colony rearing. The Hessian fly colony used for this study was collected from wheat fields in Scott County, KS in 2005 and subsequently identified as biotype Scott-KS-GH-05 (Chen et al. 2009b, Chen et al. 2009a). The colony was reared on Karl 92, a Hessian fly susceptible wheat variety, in greenhouse conditions of 22°C and 16:8 h (light:dark) photoperiod. All Hessian flies used in the bioassays were newly eclosed (<12 h) the previous night or the morning of each bioassay replicate.

Ambient light experiment. Both male and female Hessian fly attraction to LEDs in the presence of ambient light was tested in a four-leaf clover shaped arena described by Snyder et al. (2016) (Fig. 5.1). Individual LEDs were positioned within a collection cup located at the apex of each half circle. Arena construction dictated four LED choice options, and we selected two green LEDs to fill all four collection cups. Thus, two green LEDs were used (525 nm, 16 W/m²) (Part #: C503B-GCS-CY0C0791, Cree, Inc.; Durham, NC), along with one white (16 W/m²) (Part #: SLA560WBC7T3, Rohm Semiconductor Co. Ltd.; Kyoto, Japan) and one blank cup. Green LED wavelengths were selected based on previous results from Schmid et al. (2017), which demonstrated significant attraction of male and female Hessian flies to LEDs with a wavelength of 525 nm and intensity of 16 W/m². The white LED and the blank collection cups were included as positive and negative controls for the presence and absence of light, respectively.

Newly eclosed Hessian flies (n = 18 – 20 flies/replicate) were collected using a mouth-operated aspirator the morning of each replicate, 0700 h, and sexed with a 35X stereo

microscope (Leica EZ4, Leica Microsystems Inc.; Buffalo Grove, IL). Flies were released into the center of the arena at 0730 h, and allowed 1 h to acclimate to the arena environment before exposure to the LEDs. Once LEDs were powered, the flies remained in the light arena for 23 h to allow sufficient time for individuals to make a choice. After 23 h, collection cups were removed, and the number of individuals in each cup and individuals that remained in the center of the arena (i.e., flies that did not make a choice) were quantified. The arena was housed within a chamber to control ambient light levels. Males and females were run in separate trials. A bioassay, ($n = 3$ reps) in which no ambient light was permitted to enter the arena was conducted first. Likewise, another bioassay ($n = 5$ reps) was conducted in the presence of ambient light, provided by a white LED light bar (310 lm) (UCF plug-in LED light fixture 10447, General Electric Company; Boston, MA) hung 67.3 cm above the center of the arena. While the intensity of the LED light bar was far less than the intensity of direct sunlight experienced on a sunny day, the dimensions of the light bar lent itself to easy installation above the area. We thought it prudent to begin examining the effects of ambient light with a low intensity light source, and then build to higher intensities of ambient light if Hessian flies demonstrated attraction to color LEDs.

Data analysis. Each replicate took 24 h to complete and because only one arena was available, only one replicate could be completed in a day. Therefore, the experiment was blocked through time, with each replicate (day) representing a block. LEDs were randomly assigned to collection cups for each block, making the experiment a complete randomized block design. In this design, each LED was considered a separate treatment; thus, the two green LEDs were analyzed separately for Hessian fly attraction, along with the white and blank LEDs.

Due to unequal sample size between replicates caused by flies escaping during transfer to the arena, proportion fly response to LED treatments was calculated to standardize results between replicates; however, by proportioning the data of Hessian fly response, the distribution of results was restricted between 0 and 1. Consequently, the female bioassay in the absence of ambient light was the only bioassay that failed the assumption of normal distribution for analysis of variance (ANOVA). Proportion fly response was calculated by dividing the number of flies captured in a given collection cup by the total number of flies that were captured in all collection cups. Non-responsive flies were not factored into the proportion fly response per LED treatment. The mean proportion of non-responsive Hessian flies was low for most of the bioassays (0.07 and 0.16 absence of ambient light, female and male, respectively; 0.17 and 0.41 presence of ambient light, female and male, respectively). Proportion of flies responding to LED treatments was compared using one-way analysis of variance (ANOVA) (R 3.4.1), using a general linear model for the fixed effect of LED. Day was included as a blocking factor in the model because only one replicate could run per day, owing to our access to only one light arena. Means were separated using Tukey-Kramer test with statistical significance set at $\alpha = 0.05$.

Wheat odor experiment. Binary-choice bioassays were performed in Y-tube arenas (Fig. 5.2) to assess Hessian fly attraction to a green LED (525 nm, 16 W/m²) in the presence of wheat odor. Y-tubes were constructed from polyvinyl chloride (PVC) tubes with an inner diameter of 3.5 cm, arm lengths of 26 cm, and a stem length of 33 cm. Air was pulled through the Y-tube from the stem at a rate of 1.8 m/s; however, air flow varied slightly during each 23 h replicate due to fluctuations in air compressor flow.

Wheat odor was introduced into a Y-tube arm by placing an individual potted wheat plant (Karl 92), 2-3 leaf stage, into a chamber attached below the arm of the Y-tube. The plant

chamber was attached under the arm of the Y-tube to prevent Hessian flies from seeing the plant during the bioassays. Wheat was grown under the same conditions as the wheat used for colony rearing, except individual wheat plants were grown in containers that fit into the Y-tube chamber to avoid damaging or uprooting plants before the experiment. Air entered the Y-tube through activated charcoal, to screen out unwanted odors, from the bottom of the plant chambers then flowing over the wheat plant before moving to the stem of the Y-tube. Identical plant chambers were attached to each Y-tube arm, and matching plant containers comprising only of soil were placed into the opposing chamber of the Y-tube. The same green LED bulb used in the LED arena bioassay was positioned in the center of the distal end of the Y-tube arm.

For each replicate of Y-tube arena bioassays, newly eclosed Hessian flies ($n = 8 - 10$ flies/replicate) were removed from lab colony at 0700 h using a mouth-operated aspirator and sexed with a 35X stereo microscope. Males and females were run in separate trials. Flies were transferred into a chamber at the end of the Y-tube stem at 0730 h, and locked in the chamber for 1 h to allow time to acclimate to Y-tube environment. Attractants (green LED and wheat plant) were placed in the appropriate Y-tube arms at 0830 h, and then flies were released from the chamber at the end of the Y-tube stem. Flies remained in the Y-tube for 23 h to allow individuals sufficient time to make a choice. A choice was defined as movement by an individual 10.3 cm up one of the arms. Each arm of the Y-tube contained a mesh funnel 10.3 cm up the arm, and Tangle-Trap Sticky Coating (The Tanglefoot Company; Grand Rapids, MI) covered the inside of the arm tube on the distal side of the funnel to prevent flies from moving to other areas of the Y-tube after making a choice.

Two preliminary bioassays ($n = 3$ reps/bioassay) were performed to ensure flies were responding to the attractants, green LED and wheat odor. Preliminary bioassays performed were

LED versus blank (nothing placed in the arm) and wheat odor versus blank (only plant container with growing medium placed in the plant chamber). After completion of the preliminary bioassay, a treatment bioassay ($n = 6$ reps) was performed consisting of LED versus wheat odor.

Data analysis. Due to unequal sample size between replicates, proportion fly response was calculated to standardize results between replicates; however, by proportioning the data of Hessian fly response, the distribution of results was restricted between 0 and 1. Consequently, some of the bioassays failed the normality distribution assumption for analysis of variance (ANOVA). Bioassays that failed the Shapiro Wilk test for normality included both the female and the male treatment bioassay LED versus wheat odor, the female control bioassays LED versus blank, and the male control bioassay LED versus blank. Proportion fly response was calculated by dividing the flies in each arm by the total number of flies released in the Y-tube for a given replicate. Proportion fly response was analyzed using ANOVA (R 3.4.1), using a general linear model for the fixed effects of green LED and wheat odor. Day was included as a blocking factor in the model replicates had to be run on multiple days, owing to limited chamber space to house Y-tube arena. Means were separated using the Tukey-Kramer test with the statistical significance set at $\alpha = 0.05$.

Field experiment. To assess Hessian fly attraction to LEDs under field conditions, an experiment was conducted within a commercial wheat field at the Kansas State University Ashland Bottoms Research Farm near Manhattan, KS during the first week of November in 2016. LED traps (Fig 5.3), consisting of PVC tubes (31 cm height, 21 cm diameter), were centrally located within a 30 m \times 30 m plot ($n = 3$). LEDs were affixed to the PVC tube in four vertical rows ($n = 6$ LEDs/row) running the height of the tube. The four LED rows faced each cardinal direction. LED treatments were randomly assigned to each plot for each replicate. LED

treatments consisted of green LEDs (525 nm, 16 W/m²), white LEDs (16 W/m²), and blanks (no LEDs). The type of green and white LEDs used were the same as the LEDs used in both laboratory bioassays.

To test Hessian fly attraction to the LED traps, flies were released simultaneously at 1, 5, and 10 m (n = 8 females and 8 males/release distance) from the LED trap. Due to a low number of flies emerging from the colony at the time of the experiment, flies were only released on the north side of the LED trap. To distinguish captured flies from the different release distances, flies were differentially marked with protein solutions. The three protein solutions used were 25% chicken egg white (eggbeaters® All Natural 100% Egg Whites, Conagra Brands Inc.; Chicago, IL) concentration in water at 1 m, bovine skim milk (Great Value Fat Free Milk, Wal-Mart Stores Inc.; Bentonville, AR) at 5 m, and unsweetened soy milk (Great Value Soymilk, Wal-Mart Stores Inc.; Bentonville, AR) at 10 m. Protein was applied to the flies using a medical nebulizer (Aeromist Plus HCS60004, Medline Industries Inc.; Northfield, IL), which produces a fine, fog-like mist (Hagler 1997). This method of protein application was developed specifically for mark-release-recapture studies of small insects using dispersal of parasitoids as a study model (Hagler et al. 2002), which makes this protein application method applicable for our experiment. We used fly ‘fogging’ methods described by Hagler et al. (2002), in which flies were housed within a Tupperware container and nebulizer was inserted into a 2.5 cm hole cut into the lid of the container. Flies were ‘fogged’ for 30 sec, after which the nebulizer was removed and the hole sealed by taping a plastic sheet over it. To avoid protein cross-contamination, different nebulizers and Tupperware containers were used for each protein marking solution. Flies were then transported to the field and released at the pre-determined distances described above. Two release times were selected, 0500 h (n = 2 reps) and 0700 h (n = 6 reps), but flies were never

released at both times during the same replicate. These two release times allowed us to examine Hessian fly attraction to LEDs before and after sunrise (0700 h when tests were conducted). Traps remained in the field for 23 h after releasing the flies, resulting in only one replicate being run a day. Hessian flies captured on traps were quantified and then removed into individual 1250 μ L microcentrifuge tubes and stored at -20°C until individuals could be analyzed for presence of selected marker proteins.

All Hessian flies were analyzed for the presence of each protein using an indirect enzyme linked immunosorbent assay (ELISA) procedure described by Hagler et al. (2014). To remove protein antigens from the flies for analysis, tris-buffered saline (TBS), 1000 μ L, was added to each microcentrifuge tube containing a single Hessian fly and incubated for 1 h at 22°C . A 100 μ L aliquot sample of the TBS protein antigen solution was added to wells corresponding to individually sampled flies of a 96-well ELISA plate (bovine and soy milk proteins: MaxiSorp, Nalgene-Nunc International; Rochester, NY; chicken egg white: Falcon Pro-Bind™, Becton Dickinson Labware; Franklin Lakes, NJ) and incubated at 4°C overnight. Unbound antigen was then removed from ELISA plates and washed five times by emptying and refilling the wells with phosphate buffered saline (PBS)-Tween 20 (P1379, Sigma –Aldrich Co.; St. Louis, MO). Hydrogen peroxide (Vi-Jon Laboratories; St. Louis, MO), 100 μ L, was added to the wells and incubated for 15 min (22°C for egg white and soy, 4°C for milk) before washing the plate twice with PBS-Tween 20. Three hundred μ L of blocking agent (chicken egg white and soy blocking agent: 1% PBS-BSA (bovine serum albumin) (P3688, Sigma –Aldrich Co.; St. Louis, MO) in dH_2O ; milk blocking agent: 25% egg white in dH_2O) was added to wells to block any unoccupied sites for antigen attachment in the wells. Plates were incubated for 30 min (22°C for egg white and soy, 4°C for milk), then washed twice again with PBS-Tween 20. The primary antibody,

50 μ L, for the respective antigen, was added to the wells and incubated for 1 h (22°C for egg white and soy, 4°C for milk) before rinsing plates five times with PBS-Tween 20. The primary antibody for each protein were as follows: rabbit anti-chicken egg albumin (C6534, Sigma – Aldrich Co.; St. Louis, MO) primary antibody for chicken egg white, rabbit anti-bovine casein polyclonal (19036, Lampire Biological Laboratories Inc.; Pipersville, PA) primary antibody for bovine milk, and rabbit anti-soy (Trypsin inhibitor) (100-4179, Rockland Immunochemicals Inc.; Pottstown, PA) primary antibody for soy milk. Next, the secondary antibody, goat anti-rabbit IgG (A6154, Sigma-Aldrich; Co., St. Louis, MO), was added and incubated for 1 h (22°C for egg white and soy, 4°C for milk). Plates were again rinsed five times with PBS-Tween 20, and 50 μ L of TMB substrate (TMB Microwell One Component Peroxidase Substrate, BioFX Laboratory Inc.; Owings Mills, MD) was added to each well. After a 10-min incubation period at 22°C, the ELISA optical density of each well was measured using an Eon Microplate Spectrophotometer (BioTek Instruments Inc.; Winooski, VT) set at 650 nm.

Data analysis. Hessian flies serving as negative ELISA controls were collected from the colony at the beginning of the experiment. Hessian flies captured on LED traps were scored positive for the presence of the protein marker solution if the ELISA optical density reading exceeded the mean negative control reading by three standard deviations (Hagler 1997). Hessian flies testing positive for protein markers were quantified corresponding to each LED trap color, and trap capture for each LED treatment was compared using the nonparametric Kruskal-Wallis one-way analysis of variance (R 3.4.1), for the fixed effect of LED trap color, as the data for the number of captured Hessian flies did not meet the assumption of normality to perform an ANOVA. Significance for mean separation was set at $\alpha = 0.05$.

Results

Ambient light experiment. Both female and male Hessian fly attraction to LEDs was significantly different between LED treatments in the absence of ambient light (Table 5.1; Figs 5.4A, C). The majority of females were attracted to the green LEDs (0.46 and 0.51 for each green LED, or 0.97 total for both green LEDs) compared to the white LED (0.03) and the blank collection cup (0.0). Females did not simply respond to the presence of light, which was demonstrated by low capture in the white LED cup, nor was capture a result of random movement; this was demonstrated by low capture rates in the blank collection cup. While females demonstrated significant attraction to the green LEDs (97% of captured females), males did not demonstrate the same affinity for specific LED wavelengths. This result is consistent with previously documented Hessian fly male attraction to LEDs by Schmid et al. (2017). The only significant difference occurred between one green LED and the blank collection cup. Males showed no statistically significant difference between the other the green LED, white LED, or the blank collection cup (Fig. 5.4C).

Female Hessian fly LED choice in the presence of ambient light was a similar pattern to female choice in the absence of ambient light. However, females choice of LEDs was not statistically different between LED options when ambient light illuminated the arena (Table 5.1; Fig 5.4B). Female attraction for green LEDs dropped from a total of 0.97 in the absence of ambient light to a total of 0.68 (0.36 and 0.32 for each green LED) in the presence of ambient light. Also, female choice of the white LED and blank collection cup increased in the treatment bioassay, 0.14 and 0.16 respectively, compared to the preliminary bioassay, 0.03 and 0.0 respectively.

Male Hessian flies choice of LEDs was also not significantly difference between LED options in the presence of ambient light (Table 5.1; Fig 5.4D). Males choice of LEDs varied little between the four options, ranging from 0.15 to 0.38. Ambient light decreased the ability of males to discern between the LED options.

Wheat odor experiment.

Control bioassays. Two control bioassays (Figs. 5.5A, B, D, E) were performed to assess female and male attraction to the green LED and wheat odor in the Y-tube arena. A statistically significant difference was shown between the choice options (including no choice) for each control bioassay, except for the male green LED versus blank bioassay (Table 5.1). In the green LED versus blank bioassay, females chose the green LED arm 93 times more than the blank arm (Fig 5.5B). In the wheat odor versus blank bioassay, female Hessian flies chose the arm containing the wheat odor 4.4 times more than the blank arm (Fig 5.5A), while males showed no significant difference between the wheat odor arm and the blank arm (Fig 5.5D). These control bioassays demonstrated female Hessian fly attraction to the green LEDs and wheat odor in the Y-tube arena, but males were not attracted to wheat odor or green LEDs in the control bioassays.

Treatment bioassay. Both female and male Hessian flies showed a significantly higher attraction to the green LED arm than the wheat odor arm (Table 5.1; Figs 5.5C, F). Females chose the green LED arm 61.4 times more than the wheat odor arm, while males chose the green LED arm 2.0 times more than wheat odor arm.

Field experiment. Only 22 of the 1,152 (1.9%) Hessian flies released during the field study were captured on the LED traps. Of the 22 captured Hessian flies, a majority (77%, n = 17) were released 1 m from the trap, and only 9% (n = 2) and 14% (n = 3) of the captured flies were

released at 5 m and 10 m, respectively. The sex of the captured flies could not be consistently determined visually from the traps.

Trap captures showed no significant difference between LED treatments for pre-sunrise ($\chi^2_2 = 0.00$; $P = 1.00$) and sunrise ($\chi^2_2 = 1.69$; $P = 0.43$) release times (Fig 5.6). However, a difference in the average Hessian fly capture between pre-sunrise ($n = 4.50/\text{rep}$) and sunrise ($n = 2.17/\text{rep}$) releases was observed; though, it should be noted that pre-sunrise LED trapping only had two replicates.

Discussion

Laboratory experiments revealed previously undocumented behaviors of Hessian fly attraction to green LEDs in the presence of ambient light and wheat odor. Both female and male Hessian flies were attracted to green LEDs significantly more than wheat odor in the Y-tube bioassay; however, males showed no statistically significant attraction to wheat odor or the green LED in the control experiments. Ambient light also affected male and female attraction to the different LED treatments, decreasing Hessian fly capture in green LED collection cups, while increasing capture in white LED and blank collection cups. This indicated that Hessian flies may not respond to green LEDs during daylight hours. The LED trap field experiment confirmed this finding, as daily capture rates were higher for flies released before versus after sunrise, and there was no statistically significant difference between trap capture amongst the LED treatments. In the field experiment, Hessian flies were attracted to the LED traps but only from release points that were 1 m away.

The combined results of all these experiments increased our understanding of Hessian fly behavior. First, females chose green LEDs significantly more than wheat odor. Previous work analyzing female oviposition behavior showed that female Hessian flies respond to visual cues

before switching to olfactory cues during oviposition (Harris et al. 1993). Thus, our result aligns with previous research describing female Hessian fly behavior during oviposition. It was important to confirm this behavior remains intact when LEDs are the visual stimuli, as previous research has shown that female Hessian fly attraction to LEDs can differ slightly than reflected light (Schmid et al. *In review*). Another important result of the Y-tube experiment was the lack of attraction to wheat odor exhibited by males, even when wheat odor was the only potential attractant in the Y-tube (Fig 5.5D). This behavior has not been documented in the literature. Previous male olfactory studies has focused on male attraction to the female sex pheromone (Foster et al. 1991, Harris and Foster 1991, Andersson et al. 2009), but the recent discovery of male Hessian flies attraction green LEDs (Schmid et al. 2017) reprioritized a general need to understand male Hessian fly response to additional environmental cues like wheat odor. Males of other insect species show similar behaviors, with attraction to chemical and visual cues occurring both individually and in combination (Epsky and Heath 1998, Otalora-Luna and Dickens 2011). Female Hessian flies use wheat odor to locate oviposition sites (Harris and Rose 1990, Harris et al. 1993), but it is unknown if males use wheat odor to direct movement, potentially as a means to locate host plant patches more likely to harbor females search for oviposition sites. Our results suggest that males do not use wheat odor to locate female habitat. This is in line with previous research that shows mating primarily occurs at the site of emergence and males employ the female sex pheromone to locate mates (McKay and Hatchett 1984, Bergh et al. 1990), so it would seem there is less need for males to use wheat odor to locate host plants potentially inhabited by females. Consequently, our results align with known adult male Hessian fly olfactory behaviors (i.e., no attraction to wheat odor). Though, it is interesting that males were not attracted to wheat odor, as their attraction to green light in previous studies suggests males

use visual cues associated with their host plants. While males use visual cues associated with host plants during taxis (although this response does not mean that it is biologically meaningful), the results of our Y-tube bioassay demonstrates that males do not use olfactory cues from host plants; instead rely primarily on the female sex pheromone.

The LED arena experiment revealed ambient light decreased Hessian fly capture in green LED collection cups, while Hessian flies captured in white LED and blank collection cups increased in the presence of ambient light. These results show that ambient light affected Hessian fly attraction to green LEDs. The LED trap field experiment confirmed the results of the laboratory LED arena experiment. Daily capture rate of flies released before sunrise was higher than after sunrise and there was no statistically significant difference between trap capture amongst the LED treatments in the field, despite LED wavelength and intensity remaining the same in the presence and absence of ambient light. However, ambient light does reduce the contrast between the LEDs and the surrounding background, changing the background from dark or black to light. Contrast is the difference in luminance or color that distinguishes objects within a field of view. Contrast has been shown to affect female Hessian fly approach and landing on paper targets (Harris et al. 1993). Therefore, it is plausible that the reduced contrast in the LED arena when ambient light was present affected female approach and therefore attraction to the LEDs. The effect of contrast on male Hessian fly phototaxis has not been previously studied, but the results of the LED arena experiment indicate that contrast may be important for male Hessian fly attraction to LEDs as well and requires further research.

Reduced attraction to green LEDs in the presence ambient light can alter LED field trap deployment strategies because temporal activity cycles of adult Hessian fly overlap with daylight hours (Harris and Rose 1989, Bergh et al. 1990, Harris and Rose 1991). Post emergence, male

activity (walking and flying) is motivated by finding mates, typically occurring between 1900 h – 1100 h; whereas females begin calling for males soon after emergence (0100 h – 0900 h), typically within ≈ 9 min when males are present (Bergh et al. 1990). After copulation, female behavior switches to oviposition site selection (Harris and Rose 1991), during which females can cover a significant area, 660 m² in 2 h in host patches and 1,500 m² in 2 h in non-host patches (Withers et al. 1997). However, the movement of males throughout the landscape is less understood, knowledge of the distance males react to calling females or dispersal of males post-mating would be important to understand the potential for LEDs to attract males under field conditions. Even though both sexes are active after sunrise, their daily activity cycle begins before sunrise, which means that visual cues like green LEDs would be a useful tool to attract Hessian flies before sunrise. Also, understanding that ambient light decreases Hessian fly attraction after sunrise is important for energy conservation in trap design (e.g., LEDs can be turned off after sunrise to conserve battery power). This information is critical to development of trap design and application of traps in a monitoring strategy.

The distance Hessian fly are attracted to LEDs in the field is another important aspect of Hessian fly behavior that should be considered when developing a Hessian fly monitoring program utilizing visual attractants. The field experiment shows the majority (77%) of Hessian flies captured were released 1 m from the trap; consequently, a large number of LED traps would be required to monitor for Hessian fly throughout an entire commercial wheat field. However, there are several methods to increase the efficiency of trap deployment strategies utilizing visual cues. First, understanding Hessian fly distribution within fields and environmental factors affecting Hessian fly distribution can inform implementation of monitoring strategies (e.g., placement of traps in areas of the field most likely to incur a Hessian fly infestation). Second,

LED trap design could be modified to have more LEDs set to higher intensities, or monitoring could also be modified to enlarge the size area encompassed by the visual attractant. Third, contrast with background may also be important for Hessian fly attraction to LEDs. Future studies focusing on these topics are needed to improve the efficiency of monitoring for Hessian fly with visual cues under field conditions.

Ultimately these experiments contribute to our understanding of how environmental elements affect male and female Hessian fly attraction to visual cues, i.e., green LEDs. It is important to add further understanding to male response to environmental cues. While these experiments show that ambient light decreases Hessian fly attraction to green LEDs, Hessian fly adult activity before sunrise makes it plausible for LED traps to capture flies before sunrise. Together these results contribute to understanding Hessian fly behavior with regard to monitoring, but did not result in a strong recommendation for improving the monitoring strategy.

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Tables and Figures

Table 5.1 ANOVA results for laboratory experiments examining male and female Hessian fly attraction to green LEDs (525 nm, 16W/m²) in the presence of ambient light and wheat odor.

Laboratory Bioassay	df	F	P-value
<u>LED arena</u>			
Males			
Ambient light absent	3, 7	4.64	0.04
Ambient light present	3, 15	2.74	0.08
Females			
Ambient light absent	3, 7	29.28	≤0.01
Ambient light present	3, 15	2.94	0.07
<u>Y-tube</u>			
Males			
Wheat odor vs. Blank	2, 5	96.50	≤0.01
Green LED vs. Blank	2, 5	4.92	0.07
Wheat odor vs. Green LED	2, 14	77.81	≤0.01
Females			
Wheat odor vs. Blank	2, 5	37.05	≤0.01
Green LED vs. Blank	2, 5	76.25	≤0.01
Wheat odor vs. Green LED	2, 14	1530.00	≤0.01

Figure 5.1 Schematic of the bioassay light arena (view from above) used to test Hessian fly attraction to LEDs at varied wavelengths and intensities.

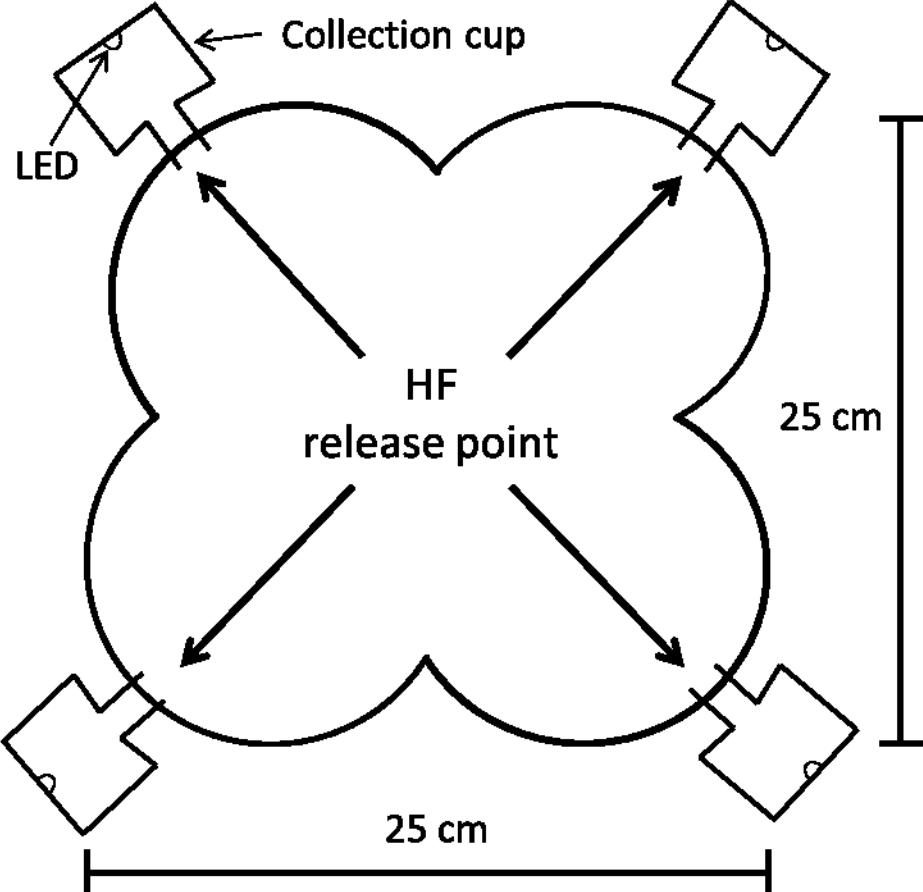


Figure 5.2 Schematic of the Y-tube arena A) view from above and B) view from the side used to compare Hessian fly attraction to LEDs and wheat odor.

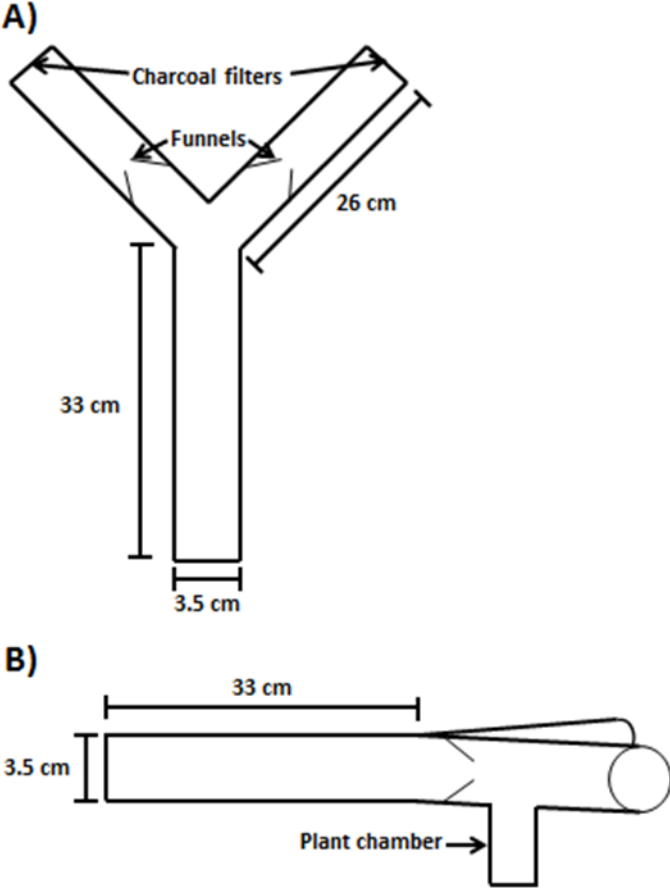


Figure 5.3 Schematic of the LED trap used to test Hessian fly attraction to LEDs under field conditions.

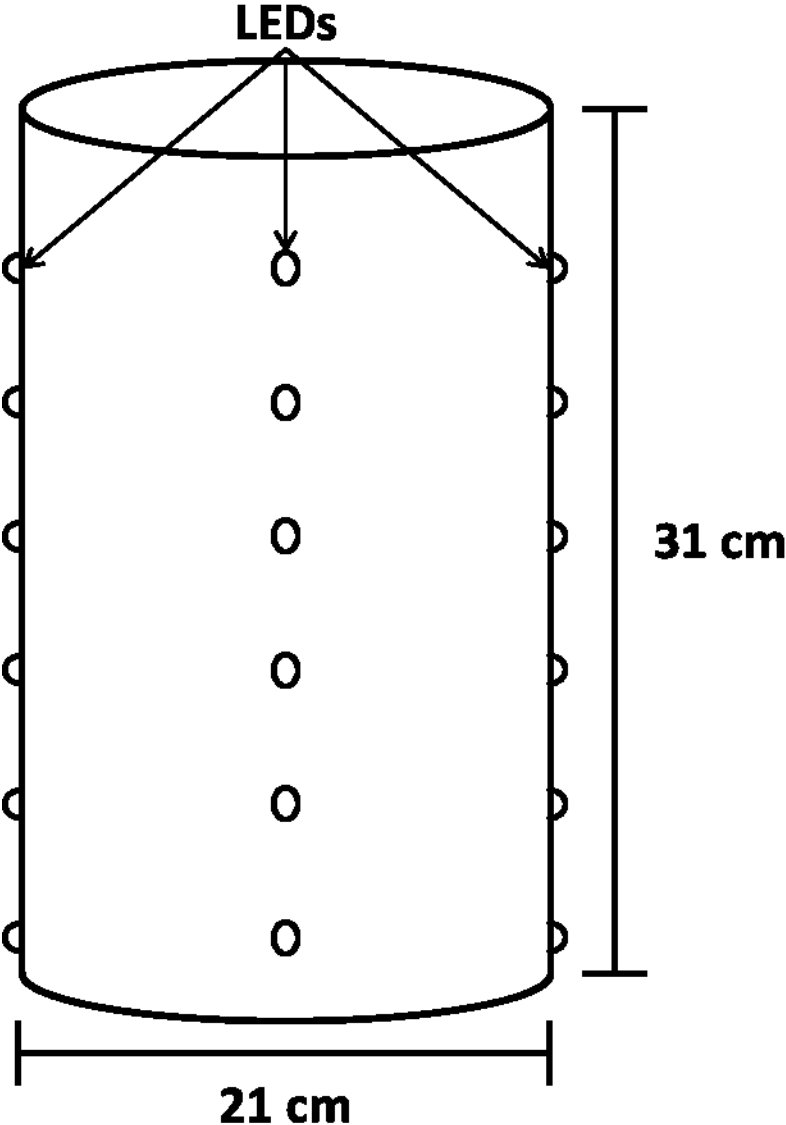


Figure 5.4 Mean percentage (+ SEM) of female (A – B) and male (C – D) Hessian fly LED choice in the absence of ambient light (A, C) and in the presence of ambient light (B, D). Statistical difference between LED treatments is signified by different letters above the bars ($\alpha = 0.05$).

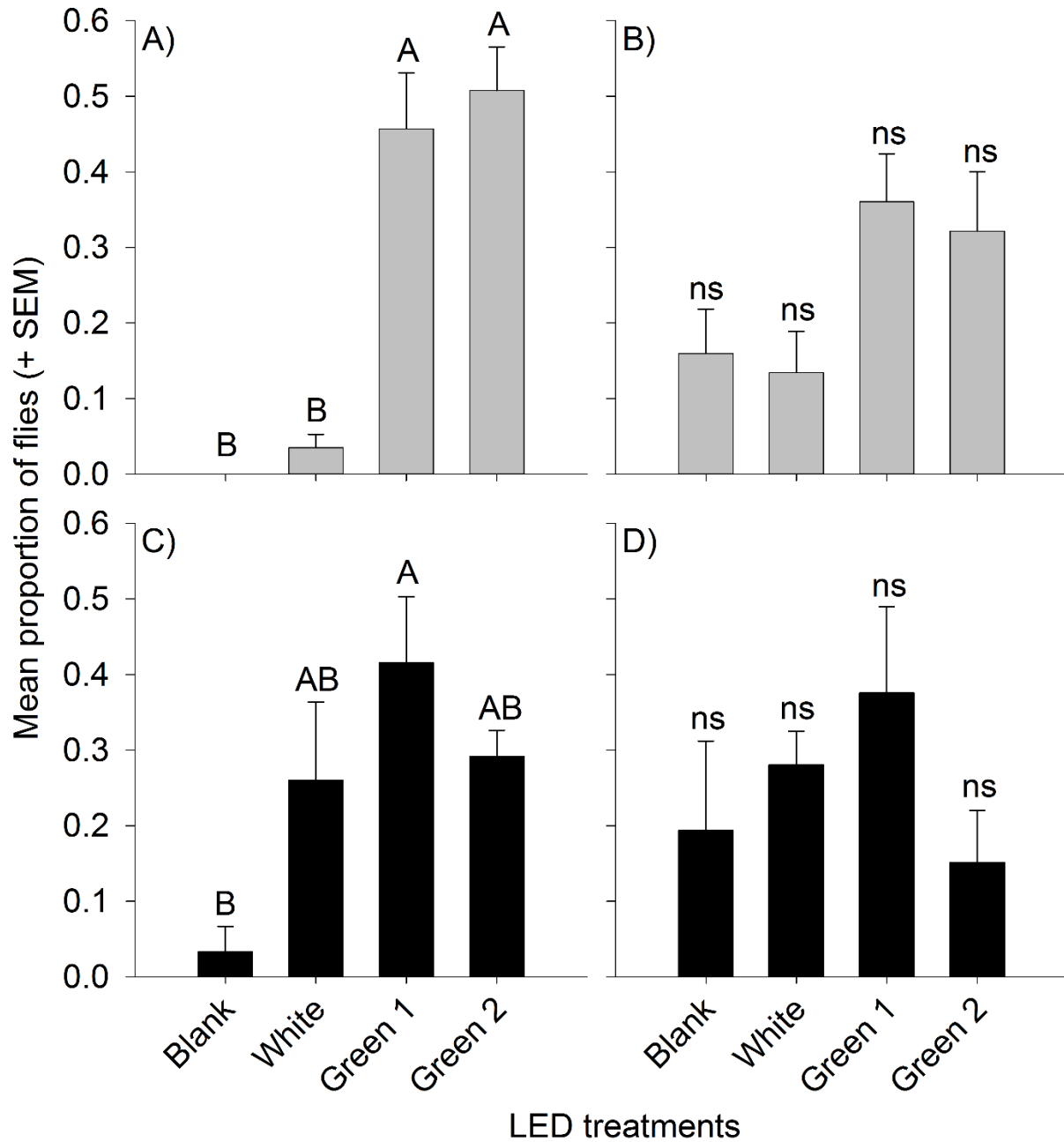


Figure 5.5 Mean percentage (+ SEM) of female (A – C) and male (D – F) Hessian fly choice in Y-tube experiments examining Hessian fly attraction to green LED (525 nm, 16 W/m²) and wheat odor. Statistical difference between treatments is signified by different letters above the bars ($\alpha = 0.05$).

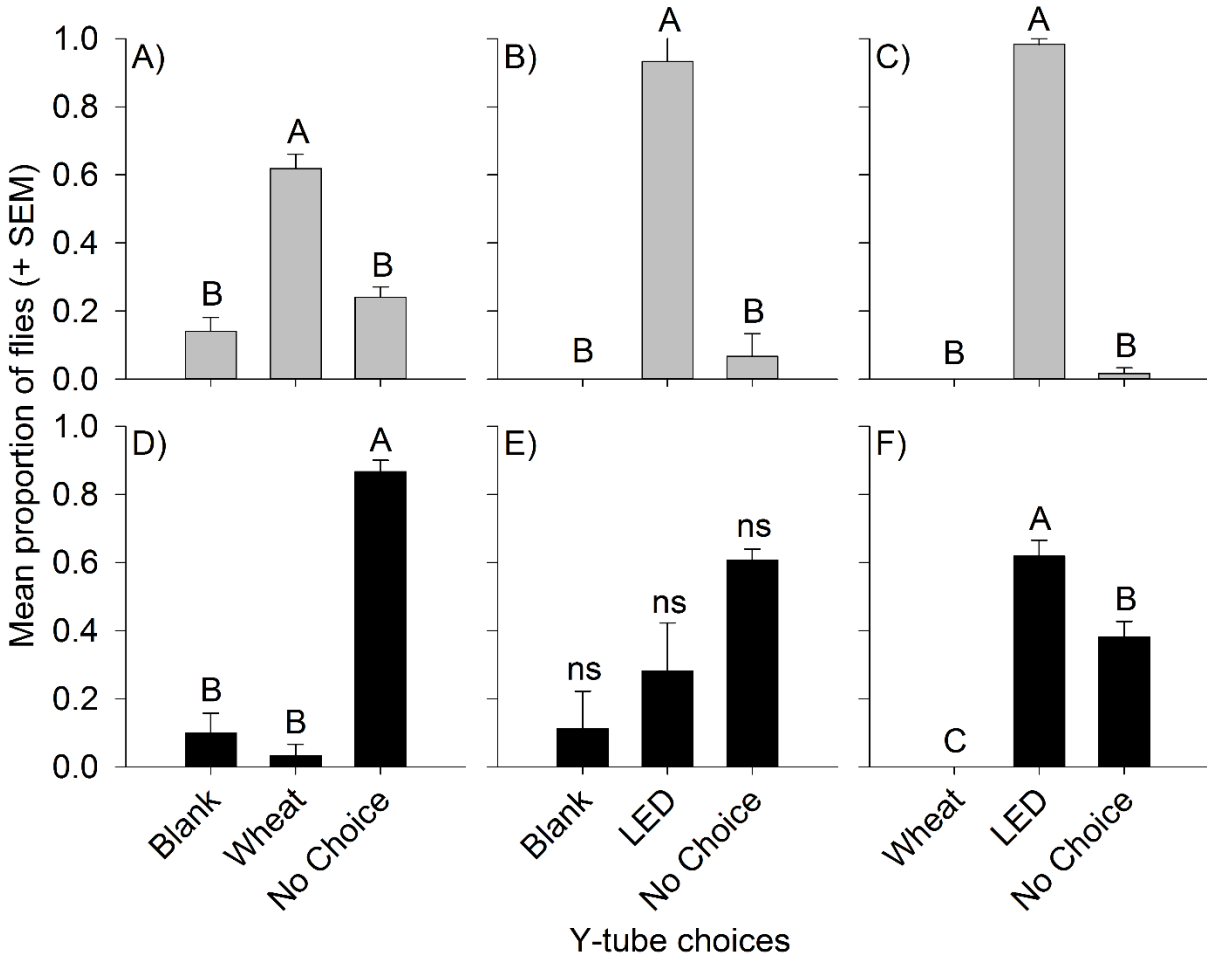
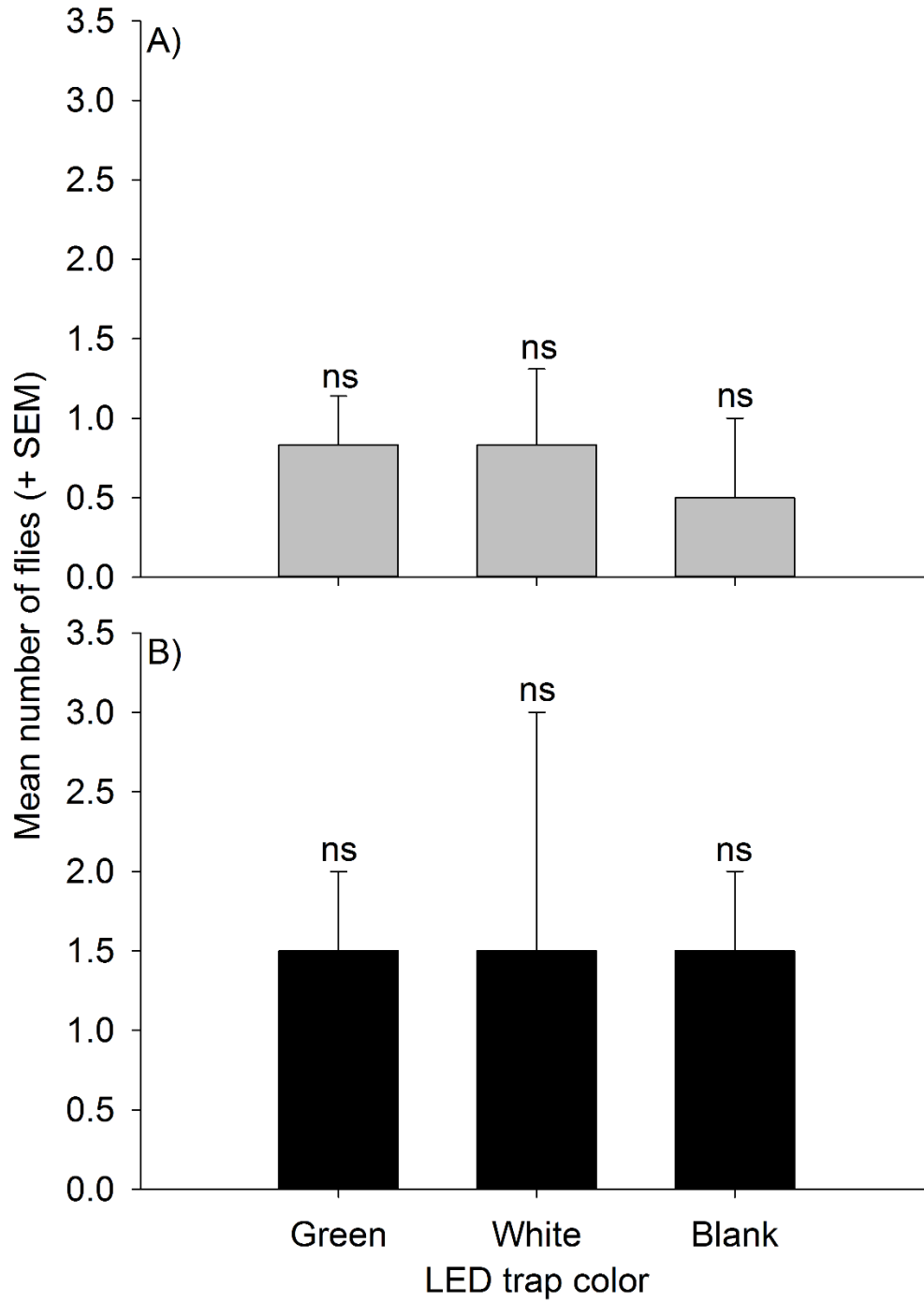


Figure 5.6 Mean percentage (+ SEM) of female (A) and male (B) Hessian fly capture on traps containing green LEDs (525 nm, 16 W/m²), white LEDs (16 W/m²), and no LEDs (blank). No statistical difference of mean number of flies captured on the different LED color treatments was found ($\alpha = 0.05$).



Chapter 6 - Hessian Fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), Attraction to Green Laser Light Display

Introduction

Insect movement is directed by a set of behaviors that are influenced by both external and internal stimuli (Triplehorn and Johnson 2005, Whitfield and Purcell III 2013). Insects can utilize a wide range of external sensory stimuli to influence their movement patterns, utilizing olfactory, gustatory, auditory and visual systems (Southwood 1962, Johnson 1969). Humans take advantage of these factors governing insect behavior to help mitigate insect pest damage. Use of volatile chemical stimuli such as pheromones and kairomones in traps is a commonly used tactic that exploits insect behavioral response to increase trap capture efficiency (Metcalf and Metcalf 1992, Witzgall et al. 2010). Another approach that exploits attractants for pest management is the establishment of areas within a field or adjacent to a field that serves as an attractant, such as a trap crop (Shelton and Badenes-Perez 2006) or that attract beneficial insects to the fields and enhance pest control (Morandin et al. 2014, Inclan et al. 2016).

Attractants can be both natural resources (e.g., trap cropping, kairomones) or artificial attractants (e.g., synthetic pheromones and kairomones, light traps) (Hokkanen 1991, Foster and Harris 1997). Artificial attractants are often exaggerated imitations of specific elements found in the environment (Ladd and Klein 1986, Sternlicht et al. 1990, Arn et al. 1992). For example, female apple maggot flies, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), use a combination of odors and visual stimuli from host apples to locate oviposition sites (Prokopy and Hauschild 1979, Aluja and Prokopy 1993), which led to the production of apple maggot fly traps comprising of specific synthetically produced apple odors and red spheres slightly larger than an

apple (Prokopy 1975, Fein et al. 1982). Artificial stimuli, such as chemical compounds (e.g., insect pheromones), plant kairomones, and insect-food supplements, can also be joined with natural traps (e.g., trap crops) to increase effectiveness (Hagen et al. 1970, Hardee 1982, Metcalf 1985, Hokkanen 1991). These combinations can work in different ways, either the combination of attractants offers a greater response or the different attractants work over different spatial distances or through different mechanisms (e.g., attractants and arrestants).

Female Hessian flies, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), use a combination of multiple cues (visual, chemical, and tactile) from host plants (e.g., wheat) during oviposition site selection (Bergh et al. 1990, Harris and Rose 1990, Harris et al. 1993). Currently developed Hessian fly monitoring traps utilize the Hessian fly female sex-pheromone lure to attract males (Schwartz et al. 2015, Knutson et al. 2017). This trap is effective at detecting low densities of males, but trap captures have not correlated with larval infestations in the surrounding area (e.g., wheat field) (Knutson et al. 2017). Monitoring methods for the Hessian fly could benefit from a method that uses artificial visual stimuli to enhance environmental cues, especially to improve monitoring for Hessian fly larval infestation. Female oviposition behavior initially relies on visual (plant color and shape) and chemical stimuli to direct females into a 1 cm zone surrounding a potential host before relying on tactile and chemical cues from the potential host to initiate settlement and oviposition (Harris and Rose 1990, Harris et al. 1993). Previous research has shown that female Hessian flies are also attracted to artificial light sources (i.e., light emitting diodes (LEDs)) at the lower end of the green spectrum (502 – 525 nm) set to high intensities (16 W/m²) (Schmid et al. 2017). However, the use of LEDs is limited owing to their inability to attract Hessian flies under field conditions (Schmid et al. *In review*). A more efficient technique to use light as a monitoring tool for Hessian flies under field conditions may

be to switch from a point light source, like LEDs, to an applied light source that can be combined with natural resources needed to complete settlement and oviposition of Hessian fly. An applied light source can enhance the initial approach of females during oviposition by applying light to host plants, while retaining the required chemical and tactile features of the plant to complete oviposition site selection.

Laser projection displays, typically used for holiday displays on the exterior of buildings, are a new commercially available product that creates visual targets in a variety of colors. Integrating a green laser display could potentially enhance visual stimuli (i.e., green light previously shown to be attractive to Hessian fly) in the environment, and could be used in combination with wheat plants to maintain the natural visual, chemical, and tactile cues needed to trigger female settlement and oviposition. Using a green laser display to enhance visual stimuli would potentially concentrate Hessian fly oviposition to the area covered by the laser display. Concentrating Hessian fly infestation could help with monitoring of Hessian fly pupae, as previous research has shown Hessian fly pupal infestations are heterogeneous in wheat fields (Schmid et al. *In review*). Therefore, the objective of this study was to examine Hessian fly movement and oviposition site selection in areas of wheat covered by a green laser display.

Materials and Methods

Colony rearing. Hessian fly colony used for this study was collected from wheat fields in Scott County, KS in 2005, of biotype Scott-KS-GH-05 (Chen et al. 2009b, Chen et al. 2009a). Colony was reared on Karl 92, a Hessian fly susceptible wheat variety, in greenhouse conditions of 22°C and 16:8 h (light:dark) photoperiod. All Hessian flies used in the bioassays were newly eclosed (<12 h) the previous night or the morning of each bioassay replicate.

Laser bioassay. Hessian fly attraction to wheat reflecting green light (530 nm) produced from a laser projector (MagicPrime Laser Light, Green and Red Stars, Luckled, Inc. San Marino, CA) was performed in $346 \times 82 \times 44$ cm cages (length, width, and height). Bottom of the cages were lined with 18 flats containing rows of wheat (Karl 92) at the 2-3 leaf stage; flats were arranged in a 3×6 pattern (Fig 6.1). To simulate field conditions, wheat was planted with a 17.8 cm row spacing with a seed spacing of approx. 1 seed per row cm. This produced 5 rows of wheat running the length of the cages. The laser projector was centered 62 cm above Flat 17 with the laser light display pointed directly down on the wheat. This resulted in reflectance of the laser display on the plants in 6 flats (3×2 pattern, or Flats 13-18) (Fig. 6.1). A 6 cm diameter hole was cut in the top of each mesh cage to allow laser lights an unimpeded path to the wheat plants below. The laser projector was inserted into the hole and cage mesh was sealed with hot glue around the projector to prevent flies from escaping.

Flies (15 females and 5 males) were released at 0700 h on two consecutive days for each replicate ($n = 5$). Flies were released onto centrally located wheat plants in the center flat (i.e., Flat 2; Fig. 6.1) at the opposite end of the cage from the laser. Cages were housed in rearing rooms (3.8×3.8 m) to prevent ambient light from affecting the contrast of the green laser lights from the surrounding wheat. Three days after the second release of flies, wheat flats were transferred from rearing rooms to the greenhouse to continue growth and development of the plants (22.8° C, 13:11 h light:dark). This allowed Hessian fly larvae to complete development to the pupal stage (≈ 21 days after first release day). Once larvae reached the pupal stage, plants were uprooted from the soil and stored at 4° C until Hessian fly pupae could be quantified by plant dissection. The total number of pupae, number of plants infested, and number of plants per row for each flat were counted.

Data analysis. To determine the effect of the laser display on Hessian fly infestations, flats were divided into six sections (A – F) based on the distance from the laser display (Fig. 6.1). Proportion plants infested with pupae per section for each replicate were calculated to standardize unequal numbers of plants per row in each flat. The proportion of Hessian fly infested plants was calculated by dividing the total number of plants infested by the total number of plants per section. The number of pupae within each section was also quantified. A one-way analysis of variance (ANOVA) (R 3.4.1) was used to compare the number of pupae in each flat section (data was log-transformed to meet the assumption of normality), and means were separated using Tukey-Kramer test. Proportion of plants infested fit a non-normal distribution, so a Kruskal-Wallis nonparametric ANOVA (R version 3.4.1), which is applicable to non-normal data distributions, was used to compare proportion of plants infested of each flat section. A Dunn's test was used to make pairwise comparison of means. Statistical significance for P – value was set at $\alpha = 0.05$ for both the number of pupae and proportion of plants infested.

A correlation between the two metrics of Hessian fly infestation (number of pupae and proportion of plants infested) and the average number of laser points per cm^2 in each flat section was assessed with linear regression (R version 3.4.1). The average number of laser points per flat section was calculated from counts of laser points in an 11.4 cm^2 area at the center and four corners of each flat.

Results

Overall, both metrics of Hessian fly oviposition site selection were significantly different between the flat sections (number of pupae: $F_{5, 24} = 3.68$; $P = 0.01$; proportion of plants infested: $\chi^2_5 = 13.90$; $P = 0.02$) (Fig. 6.2). The number of pupae per flat section was 13.56 times greater in the section where plants were directly under the laser display (section F) compared to sections

not covered by the laser display (sections A – D). The number of pupae was also 4.28 times greater in the section E, which was covered by the laser display, though the density of laser points was less in section E (0.54 laser points/cm²) than section F (3.91 laser points/cm²). Regression analysis showed that the number of pupae was significantly correlated with the density of laser points ($F_{1,4} = 230.70$; $P \leq 0.01$; $R^2 = 0.98$) (Fig. 6.3A). Similar patterns of infestation was observed for the proportion of plants infested, with 7.17 and 3.30 times increase in sections F and E, respectively, compared to the sections not covered by the laser display (sections A – D). Regression analysis showed that the proportion of plants infested was significantly correlated with the density of laser points ($F_{1,4} = 60.44$; $P \leq 0.01$; $R^2 = 0.94$) (Fig. 6.3B).

Discussion

Both the number of pupae and proportion of plants infested were significantly higher in wheat located directly under the laser display (section F) than wheat not covered by the laser display (sections A – D) (Fig. 6.2). However, despite showing a higher number of pupae and proportion of plants infested, section E (which was also covered by the laser display) was not statistically different from the flat sections not covered by the display (Fig. 6.2). Even though section E was covered by the laser display, the concentration of green points from the laser display contacting wheat was less in section E than section F, 0.54 and 3.91 laser points/cm², respectively. The higher concentration of green points in section F may be an important factor that increased oviposition in this section of flats. Female Hessian flies utilize specific plant characteristics during oviposition site selection. Specifically, aspects of visual cues, e.g., intensity, wavelength, and spatial dimensions (i.e., greater area and increased vertical contour length), affect Hessian fly oviposition behavior (Harris and Rose 1990, Harris et al. 1993,

Schmid et al. 2017). The higher concentration of laser points in section F may have been dense enough to represent one large target area for females, resulting in more landing in the area followed by oviposition. However, cages used in experiments can have unintended effects on insects (Fox et al. 2004, Perillo et al. 2015), and the end of the cage may have stopped further female movement and concentrated oviposition in the area directly under the laser display. Future studies in more open areas will be needed to fully address how much the lights alone concentrate oviposition.

These results demonstrate potential to incorporate visual cues into Hessian fly monitoring programs. However, previous research examining incorporation of visual cues (green LEDs) into Hessian fly monitoring have shown similar potential under laboratory conditions, but laboratory results did not translate to effective monitoring under field conditions (Schmid et al. *In review*, Schmid et al. 2017). One reason for poor Hessian fly response to LEDs in the field proposed by Schmid et al. (*In review*) was the short response distance Hessian flies exhibit to LEDs. The laser displays used in our experiment offer a solution to this problem because the area covered by the display can be increased by simply increasing the height of the laser above the wheat canopy. The disadvantage of increasing the area covered by the display is decreased concentration of laser points. The results of our experiment suggest that reduced concentration of the display can result in decreased oviposition. Further work is needed examining the effect of laser point density on Hessian fly oviposition rates to determine an optimal density of laser points required to concentrate oviposition within a desired area. This information could inform deployment or design of laser displays as a Hessian fly monitoring tool.

Our results show laser displays can attract female Hessian fly from 3.27 m to oviposition sites within a caged wheat microcosm. Attracting females to a defined area to concentrate

oviposition has potential applications to improve monitoring efficiency for Hessian fly larvae, as larval infestations is heterogeneous between wheat fields (Schmid et al. *In review*, Alvey 2009). The laser displays would provide a defined area for producers to monitor for Hessian fly larvae within their fields, and could be managed according to the infestation level, with the goal of preventing infestation to spread to the rest of the field. However, the scale of production wheat fields (typical production fields being several hectares) presents a problem, as a laser display cannot be expected to be deployed throughout an entire field. Additionally, Hessian fly infestations are not uniform across the field (Schmid et al. *In review*). Therefore, laser display placement should be limited to areas most susceptible to Hessian fly infestation. Resistant varieties, adherence to local fly-free-dates, destruction of volunteer wheat 2 – 3 weeks prior to planting, and insecticidal seed treatments are management practices that reduce a field's susceptibility to Hessian fly infestation (Schmid et al. *In review*). Fields not administered these practices are more vulnerable to infestation, making them candidates for monitoring programs to mitigate infestation risk. Within susceptible fields, areas closer to volunteer wheat (either within the field or in adjacent fields) are thought to be more vulnerable to Hessian fly infestation because female flight distances are known to alter between a series of short flights among neighboring plants before switching to a single longer flight and the estimated area traveled by females during oviposition is smaller in host versus non-host patches (Withers and Harris 1996, Withers et al. 1997). This suggests that areas in closer proximity to volunteer wheat, which may harbor Hessian fly pupae, are at higher risk for infestation, which makes these areas better locations to monitor for infestation with a green laser display. Future studies are required to assess field distribution of immature Hessian flies, and examine correlation between larval

distribution and surrounding landscape cover to determine effect of volunteer wheat proximity on larval distribution.

While our results show promise for the development of a new monitoring method for Hessian fly, many questions concerning Hessian fly response to the laser display need to be answered first. Hessian fly response to the laser display under field conditions, effect of laser display concentration, and the distance females are attracted to the display are the next steps toward development of a new Hessian fly monitoring method utilizing a laser display.

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Tables and Figures

Figure 6.1. Schematic of experimental cage design used to test Hessian fly oviposition site selection in the presence of laser light display.

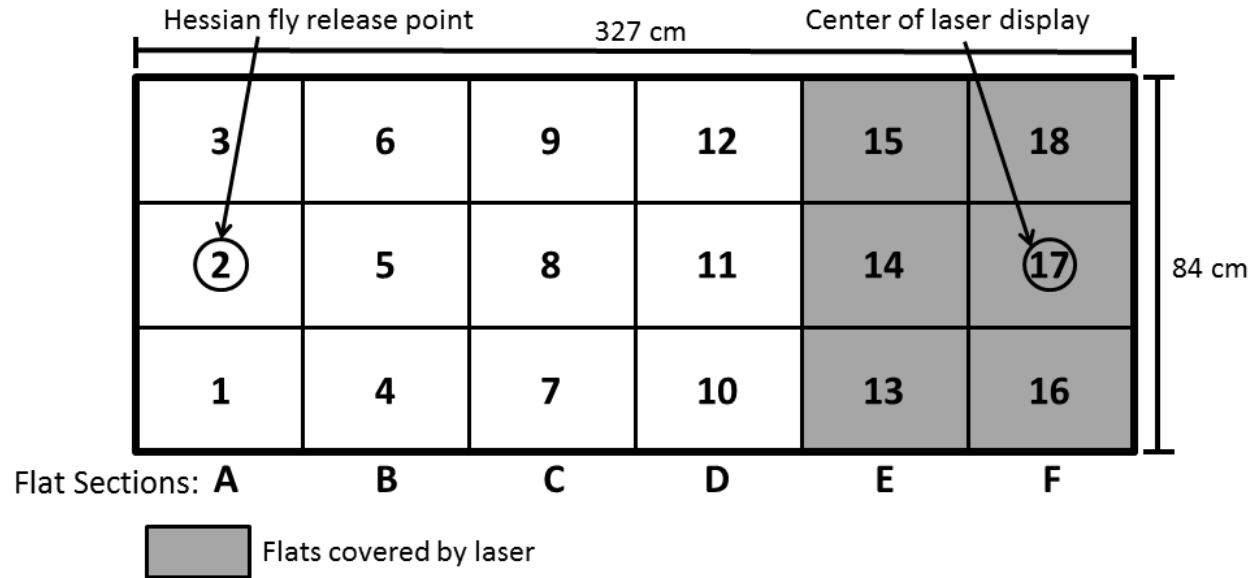


Figure 6.2. Mean number of pupae (+ SEM) 272.5, 218, 163.5, 109, 54.5, and 0 cm from green laser display, A – F respectively (A), and mean proportion of plants infested (B). Bars with same letter above are not statistically different ($\alpha = 0.05$).

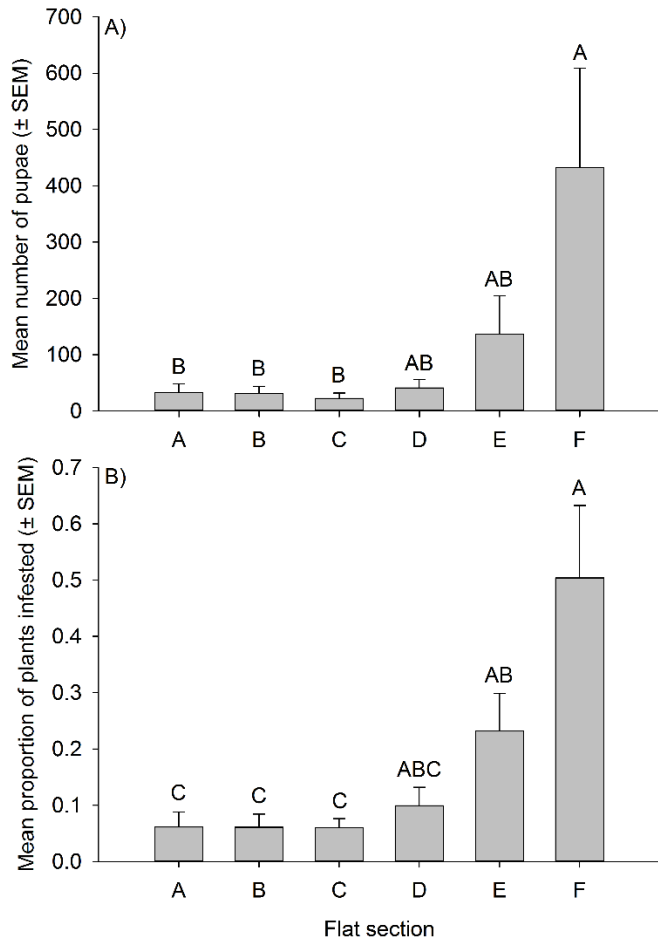
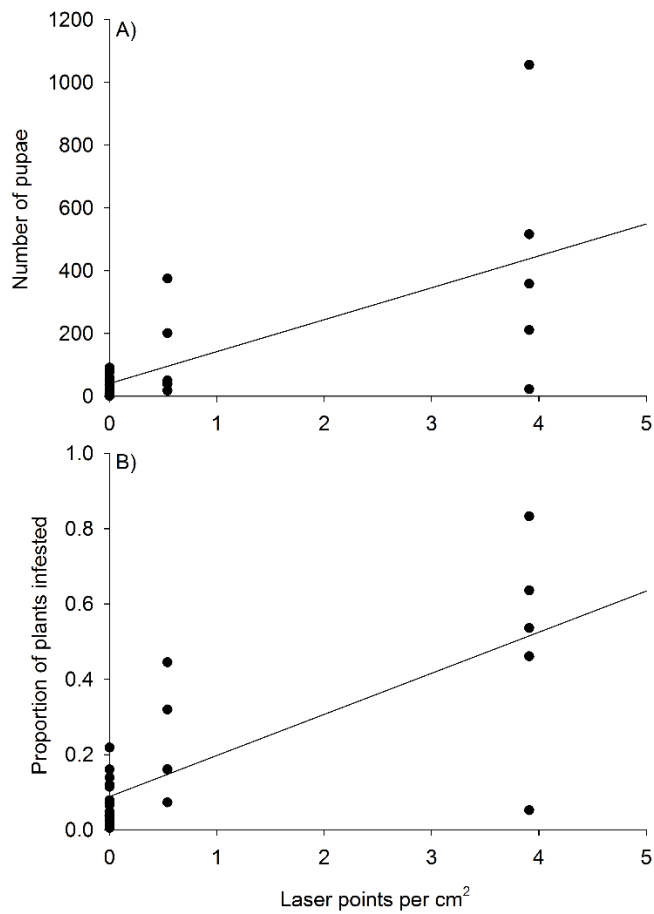


Figure 6.3. The effect of green laser point density on Hessian fly infestation (number of pupae and proportion of plants infested). (A) Number of pupae = $40.73 + 101.67 \cdot \text{laser points/cm}^2$, $R^2 = 0.98$, $F_{1,4} = 230.70$, $P \leq 0.01$. (B) Proportion of plants infested = $0.09 + 0.11 \cdot \text{laser points/cm}^2$, $R^2 = 0.94$, $F_{1,4} = 60.44$, $P \leq 0.01$.



Chapter 7 - Landscape Effects on Hessian Fly, *Mayetiola destructor* (Diptera: Cecidomyiidae), Distribution within Six Kansas Commercial Wheat Fields

Introduction

The rich soil of prairie ecosystems in the U.S. Great Plains (ranging from North Dakota south to Texas and extending west to eastern Colorado, New Mexico, and Montana) led to large-scale cultivation of prairies beginning in the late 19th century (Ramankutty and Foley 1999). Conversion of prairies in this area of the U.S. continues to this day at annual rates of 1.0 – 5.4% (Claassen et al. 2011, Wright and Wimberly 2013), driving cropland to be a dominant land-cover of the region, covering over 40% of the Great Plains since 1973 (Taylor et al. 2015). Crop production in this area of the U.S. is increasingly simplified (Aguilar et al. 2015, Fausti 2015), owing to a favorable landscape for wide scale mechanization and crop production economics. Large monoculture enterprises are the dominant crop production model in the Great Plains, with four crops [i.e., maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench), soybean (*Glycine max* L.), and wheat (*Triticum* spp. L.)] constituting approximately 69% of field crops area (USDA-NASS 2018). With of over 16.5 million hectares (i.e. 31% of the cropland) sown to wheat every year, wheat is the most widely cultivated crop in the region, making the U.S. Great Plains the largest contiguous area of low precipitation wheat production in the world (USDA-NASS 2018). In the southern Great Plains (area encompassing the states of Texas, Oklahoma, Kansas, and Colorado), winter wheat can account for over 50% of total area sown to field crops in particular states (USDA-NASS 2018), and the crop is managed as a forage and grain crop (i.e. dual-purpose) on about 3.2 million hectares (Carver et al. 2001). Dual-purpose wheat production

systems offer a more stable source of income by diversifying and producing both stocker cattle (*Bos Taurus* L.) and grain.

Monoculture systems, especially those where continuous wheat is the predominant crop, have drastically simplified the vegetation for a significant portion of the landscape of the region that was once a highly diverse prairie ecosystem (Taylor et al. 2015). Landscape simplification affects insect communities within the landscape, often manifesting in declining insect diversity and natural enemy populations, resulting in increased susceptibility to pest outbreaks for fields within the landscape (Bianchi et al. 2006, Tschamntke et al. 2012). The Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), is an important economic pest of wheat, owing to consistent low level infestations of wheat fields (Buntin 1999, Smiley et al. 2004, Watson 2005, Chen et al. 2009b). The economic importance of this pest intensifies in southern Great Plains due to increased usage of dual-purpose wheat systems, as these fields are often sown earlier than the recommended date as means to increase forage yield (Edwards et al. 2011) and earlier sowing dates often lead to increased Hessian fly pressure (Buntin et al. 1992). However, economically significant outbreaks of Hessian fly also occur in grain-only systems and can occur in localized events (Hatchett et al. 1981, Chapin et al. 1989, Alvey 2009). Yet, it is not understood where outbreaks will occur. While wheat is the preferred host of the Hessian fly (Harris et al. 2001, Chen et al. 2009a), resulting in a higher probability for infestations to occur in wheat fields, anecdotal evidence from producers suggests that it is not known why some fields incur outbreaks and neighboring fields do not.

Lack of understanding of Hessian fly distribution in the landscape makes it difficult for producers to make informed decisions to implement Hessian fly management techniques (e.g., resistant varieties, delayed sowing date, and insecticidal seed treatments) (Schmid et al. *In*

review). Despite a minimal understanding of Hessian fly distribution, it is known that adult female Hessian flies are the primary agent of dispersal through oviposition site selection (Harris and Rose 1989), and several environmental factors affect female host selection (Harris and Rose 1990, Withers and Harris 1997, Withers et al. 1997). First, movement during oviposition is directed toward features exhibiting visual, chemical, and tactile stimuli typical of grasses (Harris and Rose 1990, Harris et al. 1993). Second, Hessian fly is known to have 16 host grasses, most in the Triticeae tribe (Zeiss et al. 1993, Harris et al. 2001). The tribe Triticeae includes major cereal crops commonly planted in the U.S. such as wheat, barley (*Hordeum vulgare* L.), and rye (*Secale cereal* L.). All of these crops serve as hosts for Hessian fly hosts, but wheat as the preferred host, followed by rye, and then barley (Harris et al. 2001, Chen et al. 2009a). The composition of host and non-host patches in the landscape affects female dispersal during oviposition, increasing the estimated area traveled during a 2 h period from 660 m² to 1,500 m² from host (wheat) to non-host (oat) patches, respectively (Withers et al. 1997). Third, the proximity of Hessian fly reservoirs (e.g., volunteer wheat, wheat stubble, and other host grasses) to a wheat field can affect female dispersal, as females alter flight distances over time, switching between a series of short flights among neighboring plants to a single long flight (Withers and Harris 1996). While it is known that these environmental factors affect female dispersal, their effect on Hessian fly distribution has not been studied.

When the dispersal of adult female Hessian fly is considered in the context of the simplified landscape of the Great Plains, it is easy to hypothesize how the landscape of the region can affect where Hessian fly outbreaks occur. Thus, our hypothesis was that the vegetative cover surrounding a wheat field would affect distribution of Hessian fly infestation within the field and between fields. Specifically, the presence of host wheat (both actively

growing and stubble) surrounding a field would increase the level of infestation in areas of the field. Therefore, the objective of this study was to examine Hessian fly distribution within commercial wheat fields and examine environmental factors that may affect their distribution.

Materials and Methods

Study area. Study fields were located in Marion and Dickinson counties, Kansas. Field sites ($n = 6$) were selected based on reported Hessian fly infestations, which occurred during the fall of 2016. Presence of Hessian fly pupae was confirmed February 2017 before sampling began. Fields ranged in size from 6.1 ha to 44.6 ha. Aspects of field histories pertinent to Hessian fly infestations such as variety, volunteer wheat control, and previous planted crop are described in Table 7.1.

Sampling methods. Sampling points in each field were laid out in a predetermined grid pattern using ArcGIS (Esri, Redlands, CA). The number and spacing of sampling points within a field were based on field size and shape, which resulted in a sampling intensity of 0.85, 1.66, 2.33, 1.58, 1.51, and 5.57 points sampled/ha in fields 1 – 6, respectively. At each sampling point, a 1 m row of plants (top 5 cm of roots and above ground material) was removed from the soil by first loosening the soil around the wheat with a potato spade and then separating plants from the soil. Plants were placed into a 22 oz. plastic bag, and labelled with sampling point corresponding to the geocoordinate. Bags were stored at 4°C to maintain Hessian flies in the pupal stage until examination for pupae within each plant could occur. After samples were gathered from all six fields, plants were dissected for the presence of pupae by peeling back leaf sheath to the base of the plant for each leaf. The total number of pupae and number of plants infested were quantified for each sampling point. Each of these metrics were used to describe Hessian fly infestation as

both Hessian fly pupae abundance and number of plants infested at each sampling point. Cover of landscape immediately surrounding the sampled fields was also recorded at time of sampling.

Spatial distribution model. Generalized additive models (GAMs) were used to understand the within field and between field variability in the spatial distribution of Hessian fly pupae abundance and the number of wheat plants infested (Wood 2017). In addition to statistical inference about the variables influencing the spatial distribution of Hessian fly pupal abundance and number of plants infested, GAMs are well-suited to predict pupae abundance and infestation rates at locations within fields that were not sampled. GAMs are a flexible approach that can incorporate variables such as plant resistance (e.g., Table 7.1) and landscape-level habitat as covariates (independent variables), but can also account for the spatial autocorrelation generated by complex spatio-temporal processes (e.g. dispersal) (Hefley et al. 2017c, Wood 2017).

Similar to generalized linear models (Wood 2017), GAMs require a distribution for the response variable (e.g., pupae abundance at a sample point). For each sample point, a negative binomial distribution was assumed for pupae counts and a quasi-binomial distribution for the number of plants infested. We assumed a negative binomial distribution for pupae counts because this response variable is restricted to non-negative integer values (i.e., $0, 1, 2, \dots, \infty$) of which there is no reasonable upper bound to assume for the counts, and because a small scale spatial aggregation (clustering) of pupae was expected, which would result in overdispersion (Pielou 1969). Similarly, a quasi-binomial distribution was assumed for the number of plants infested because this response variable is restricted to non-negative integer values, but the upper bound on the number of plants infested is the number of plants within each 1 m sample point. Like pupae counts, we expect small scale spatial aggregation of the number of plants infested,

thus quasi-binomial distribution was used, rather than a binomial, to account for overdispersion generated by this process.

The GAM framework allows for linear effects of covariates such as field characteristics as well as nonlinear effects that are captured by basis functions (e.g., spatial location). To explain the within field spatial distribution of pupae abundance and the number of plants infested, the linear effect of minimum distance from each sampling point to the field edge, wheat cover, and grassland cover adjacent to fields were included as covariates in our GAMs (Table 7.1). As an initial hypothesis, the minimum distance to edge was expected to influence the response variables (pupae abundance and number of plants infested) because female Hessian fly decrease movement for oviposition sites once inside a patch of host plants (Withers et al. 1997). Although the minimum distance to edge types was expected to influence the response variables, the effect should depend on resistance level. For example, the distance to nearest grass edge may influence the number of pupae at a sampling point, but for this effect to fully materialize the wheat cultivar sown within the field must be not be resistant to Hessian flies. As a consequence, the interaction effects of cultivar resistance (intermediate or high susceptibility) and minimum distance to edge types were included as covariates in our GAMs.

To explain the between field variability in the spatial distributions of pupae abundance and the number of plants infested, we included the level of wheat cultivar resistance scores (converted to intermediate or high susceptibility for resistance scores 5 and 9, respectively) and the previous crop at each sample point as covariates in our GAMs (Table 7.1). With the exception of field 1 (see Figs. 7.1, 7.2), wheat cultivar resistance and previous crop were constant within a field but different across fields, thus offering one possible explanation of the between field variability in the spatial distributions. In addition, to cultivar resistance and

previous crop, three landscape covariates were included: 1) elevation, 2) the proportion of winter wheat within a circular region with a radius of 1 km that encompasses each sample point hereafter referred to as “1 km buffer”, (see Fig. 7.5) and 3) the proportion of grassland within a 1 km buffer. The size of the buffer (1 km) was chosen based on anecdotal speculation from producers that Hessian fly disperse a short distance (< 1 km) in the landscape; however, the maximum dispersal distance of Hessian fly has not been documented in the literature. The proportion of winter wheat and grassland from the previous growing season within the 1 km buffer was calculated from CropScape as the proportion of 30 m x 30 m cells with a value of 24, 26, 225, 236, 238 for wheat and 176 for grassland (USDA 2016).

Similar to the within field covariates (e.g., distance to edge), we expect that the wheat cultivar must be not be highly resistant to Hessian flies for the landscape covariates to influence the spatial distributions, thus only the interaction effects of cultivar resistance and the three landscape-level variables were included as covariates in our GAMs. Although all three landscape level variables may vary within a field (e.g., elevation changes from one sampling point to another within a field), the variability within a field is minor compared to the variability between fields (see Table 7.1); thus, the landscape covariates have the potential to explain between field variability.

Not all the spatial variability of number of Hessian fly pupae and plants infested were explained by the within field and between field covariates (i.e., spatial autocorrelation; see Table 1 in Hefley et al. 2017c). Therefore, a thin plate regression spline basis function was included in our GAMs. Briefly, thin plate regression splines are a type of basis function that can be used to model the “smooth” but nonlinear effects of spatial location, which can account spatial autocorrelation (Wood 2017).

Determining which covariates influence the spatial distribution of any organism can be a challenge for two reasons. The first challenge is that the covariates of interest are often correlated. For example, minimum distance to nearest wheat edge and proportion of wheat within a 1 km buffer are likely to be positively correlated. Correlation among covariates is a well-known problem in regression-type models termed collinearity (Dormann et al. 2013). Collinear covariates have the potential to make regression coefficient estimates highly variable (i.e., have a large variance) and may also result in models where inference is sensitive to small changes in model specification (e.g., the inclusions or exclusions of a single covariate may influence the inference for other covariates in a model). The second challenge is similar to collinearity, but relates to correlation between covariates that are spatially structured (e.g., minimum distance to edge) and basis vectors used to account for autocorrelation (Hodges and Reich 2010, Wood 2017). As noted in Hefley et al. (2017c), collinearity among covariates and basis vectors is a current topic of research in spatial statistics and is a difficult problem to address in applied problems, with no clear remedy (e.g., (Hanks et al. 2015, Hefley et al. 2017a, b, c, Thaden and Kneib 2017).

Given the challenges with identifying the impact of covariates on the spatial distributions, we report for the coefficient estimates for covariates obtained from the GAMs having 90% confidence intervals (CIs) that did not contain zero. However, the “statistical significance” of these results should be interpreted with caution (Hodges and Reich 2010, Hefley et al. 2017a, b, c). Although the statistical inference related to which covariates influence the spatial distributions may be challenging to determine in this study, it is important to note that that predictions obtained from the GAMs are typically accurate and not likely to be influenced by the collinearity among covariates or among basis vectors (Hanks et al. 2015).

After fitting the GAMs to both response variables (pupae abundance and number of infested plants), the relevant model assumptions were checked. During the initial stages of the analysis, alternative specifications of the GAMs were fitted (e.g., using Poisson distribution instead of negative binomial), relevant model assumptions were checked, and if needed (or possible) models were improved accordingly (Ver Hoef and Boveng 2015).

Results

Studied fields. Fields selected for this study ranged in size from 6.1 ha to 44.6 ha, and varied with regard to factors related to Hessian fly management, such as cultivar susceptibility to Hessian fly (three fields planted to highly susceptible cultivars or blend of cultivars, and three fields planted to intermediately susceptible wheat cultivars), volunteer wheat control (adopted in half of the studied fields), crop rotation (either following maize or in continuous wheat production), and elevation (Table 7.1). This created a heterogeneous group of fields utilizing commonly practiced Hessian fly management methods. It is important to notice in Table 7.1 that all fields employed some form of Hessian fly management. Fields 1, 2, and 3 rotated from maize to wheat and planted a cultivar with intermediate Hessian fly resistance (except the south portion of field 1), whereas fields 4, 5, and 6 controlled volunteer wheat at least two weeks prior to wheat sowing.

The number of pupae, plants infested, and susceptibility of wheat cultivars for all fields in this study is shown in Fig. 7.1. This data shows that both measurements of Hessian fly infestation varied greatly both within and between fields. First, fields 1, 2, and 4 had relatively high infestation levels at particular sampling points (maximum pupae per meter row: 423, 331, and 486, respectively; maximum proportion plants infested per meter row: 50, 49, and 48, respectively), while also having low levels of infestation at other sampling points (minimum

pupae: 6, 34, and 2, respectively; minimum plants infested: 2, 11, and 1, respectively). This highlights that within field Hessian fly infestation could vary substantially. Field 3 had relatively low number of Hessian fly pupae and infested plants per sample, but at a high incidence as almost every sample in the field was infested. Lastly, fields 5 and 6 had a considerably higher number of sampling points that contained no Hessian fly pupae or infested plants than the other studied fields; with the majority of sampling points in these two fields not infested with Hessian fly. Collectively, the data shows that Hessian fly infestation was heterogeneous within and between the fields selected for this study along with the susceptibility of wheat cultivars.

The covariates used in the GAMs were the same for each response variable (pupae abundance and number of infested plants) (Table 7.1). Some of the covariates of interest were highly collinear or confounded. For example, with the exception of field 1, all fields that had maize as a previous crop (fields 2 and 3) also had wheat cultivars with intermediate susceptibility, whereas all fields that had wheat as a previous crop (fields 4, 5, and 6) had wheat cultivars with high susceptibility to Hessian fly. The pairwise correlation among the field-level covariates (distance to edge, wheat edge, and grass edge) and landscape covariates (elevation, proportion of wheat and grass within a 1 km buffer) resulted in a coefficient of determination (R^2) that ranged from 0.01-0.39. Generally speaking this level of correlation among the covariates (i.e., $R^2 < 0.4$) should not cause major concern as it relates to collinearity. In contrast, some basis vectors associated with spatial effects were highly collinear with the landscape covariates proportion of grassland and elevation, reaching values of R^2 as high as 0.83.

Spatial distribution of pupae. Similar to what we observed from the data, the GAM fit to the number of pupae shows that the expected abundance varies within and between fields (Fig. 7.2A); in other words, they are a prediction of the number of pupae within the fields.

Meaning the heatmaps in Fig. 7.2A show the number of pupae we would expect to obtain if sampling had occurred throughout the fields.

Based on our statistical analysis, the spatial distribution of pupae is inversely related to the proportion of winter wheat within a 1 km buffer in the previous growing season (Fig. 7.3). The coefficient estimates from the GAM for a wheat variety with intermediate susceptibility was -8.5 (-16.4, -0.6; 90% CI) and -14.2 (-25.6, -2.8; 90% CI) for a highly susceptible wheat variety. Based on the coefficient estimates of -8.5 and -14.2 and holding all other covariates constant, we expect that as a one moves from a landscape with a proportion of wheat within a 1 km buffer of 0.30 to an area with a smaller proportion of wheat of 0.10, the expected abundance of pupae would increase 17.0 fold (4.8, 3356.7; 90% CI) for a wheat variety with high susceptibility and 5.5 fold (1.8, 39.5; 90% CI) for an intermediately susceptible wheat variety.

Spatial distribution of infested wheat. Similar to what we observed from the data, the GAM fit to the number of infested wheat plants shows that the probability of infestation varies within and between fields (Fig. 7.2B); the variability of infested wheat within a field, however, is not as striking when compared to the within field variability observed for the expected number of pupae. (cf., Fig. 7.2A to 2B). The heatmaps for the number of infested plants (Fig. 7.2B) were generated using a similar procedure as the one used to construct the heatmaps for number of pupae (Fig. 7.2A).

The spatial distribution of the probability of infestation was negatively related to the proportion of winter wheat within a 1 km buffer in the previous growing season (Fig. 7.3). The coefficient estimates from the GAM for a wheat variety with intermediate susceptibility was -7.1 (-14.0, -0.3; 90% CI) and -8.1 (-18.4, 2.2; 90% CI) for a highly susceptible wheat variety. Based on the coefficient estimates and holding all other covariates constant, we expect that as a one

moves from a landscape with a higher proportion of wheat within a 1 km buffer to an area with a smaller proportion of wheat, the probability of infestation increase substantially.

Discussion

The data from this study shows that Hessian fly infestation, as indicated by both number of pupae and plants infested, varies substantially within and between wheat fields (Fig. 7.1). Even fields planted with cultivars of intermediate susceptibility contained a high number of pupae and plants infested at sampling points throughout the field (fields 1 and 2). Possibly, fields planted to wheat cultivars with high levels of genetic resistance to Hessian fly and specifically bred for dual-purpose systems (e.g. Edwards et al. 2012) might sustain lower numbers of pupae and plants infested, but this hypothesis cannot be ascertained within the context of our study as we did not sample fields planted to these varieties. Despite our hypotheses that distance to wheat and grass edge types, and field edge would affect Hessian fly distribution within fields, only the proportion of wheat within a 1 km buffer around the fields in the previous growing season correlated with Hessian fly infestation (i.e., number of pupae and plants infested at each sampled point). The results of this study show previously unknown distribution of Hessian fly infestation within commercial wheat fields, and produce new hypotheses about Hessian fly management in agroecosystems.

The strong influence of proportion wheat cover within a 1 km radius from sampled points on Hessian fly infestations and predictability can be partially explained by agronomic variables not necessarily controlled by that particular wheat producer. For instance, a greater proportion wheat area in the surrounding landscape, if managed by other producers, could likely be related to a greater incidence of volunteer wheat in the non-growing season, which would serve as a green bridge to Hessian fly and increase its incidence (Buntin et al. 1991). This may lead

producers in those areas to adhere more strictly to Hessian fly IPM practices out of concern for potential outbreaks, as most of these practices are implemented on a calendar schedule or in response to historical crop failures (Schmid et al. *In review*); thus, reducing infestations in areas with a greater proportion of wheat. On the other hand, a lower concentration of wheat in the surrounding 1 km area could concentrate Hessian fly populations to the remaining wheat fields in the landscape, as wheat is the preferred host over other host grasses (including rye and barley) (Zeiss et al. 1993, Harris et al. 2001, Chen et al. 2009a). Since our study found Hessian fly infestations to be larger in areas with a smaller proportion of wheat, we hypothesize that the lower concentration of wheat surrounding a field is concentrating Hessian flies to the few remaining wheat fields within that landscape. However, we did not sample from other fields within the 1 km radius of our selected fields to see if infestations were higher in the other surrounding fields. Future research is needed to examine this hypothesis. This study can serve as a starting point for future research on Hessian fly distribution in the landscape, as to our knowledge, this is the first spatial assessment to show that Hessian fly distribution is affected by the wheat area planted in the previous growing season.

Documenting the distribution of Hessian fly infestation (number of pupae and plants infested per meter row) within commercial wheat fields was an important result of this study. Anecdotal evidence from wheat producers has suggested that Hessian fly infestations commonly occur in non-uniform patterns within fields, but this has not been confirmed with scientific study and published in the literature. The data from our study supports producer claims that Hessian fly infestations can be non-uniform across fields (Fig. 7.1, 7.2). Understanding that Hessian fly infestation is not equally distributed throughout a field has important implications for Hessian fly management post-sowing. As described by Knutson et al. (2017), knowing the distribution of

Hessian fly infestation within a field can affect application of crop inputs, e.g., fertilizer, fungicides, and irrigation, or livestock grazing regiments. Thus, producers can minimize crop inputs or extend livestock grazing into areas that are not economically viable to recuperate additional input costs. This can impact the management of a significant area of wheat grown on the Great Plains specifically managed for dual-purpose (i.e., forage and grain crop, potentially 3.2 million hectares) (Carver et al. 2001). Additionally, early-sown grain-only wheat fields, which often occur in western Kansas when moisture is available early- to mid-September (Holman et al. 2011), are also more exposed to Hessian fly infestations and can benefit from our findings. However, our results do not support the hypothesis that landscape cover immediately adjacent to a field (e.g., neighboring wheat or grass fields) affects the distribution of infestation within the field. This makes it difficult for producers to predict specific areas within a field at higher risk for Hessian fly infestation. Although, our study does demonstrate the possibility to develop a prediction tool of higher risk fields using the proportion of wheat from the previous growing season within a 1 km buffer. Producers could use a prediction tool like this to then focus sampling efforts to higher risk fields.

The results of this study also inform current Hessian fly management, which consists of multiple methods (e.g., resistant wheat cultivars, insecticidal seed treatments, adherence to optimum sowing dates, and destruction of volunteer wheat or “green bridges”) (Schmid et al. *In review*). These management practices are implemented before or during sowing; therefore, they are implemented preemptively before Hessian fly infestations occur. However, our results show that the intensity of Hessian fly infestations vary between wheat fields and previous research has shown similar results across regions of Oklahoma (Alvey 2009). To help producers make informed Hessian fly management decisions, a better understanding is needed of why

infestations vary between fields. Our results show that Hessian fly infestation within a field correlate to the proportion of wheat within 1 km of the field in the previous season. This suggests the previous growing season landscape surrounding a field is important to consider when implementing Hessian fly IPM. This is not to say that management practices should be halted if a large proportion of wheat surrounds a field the previous season, as destruction of volunteer wheat, i.e., the “green-bridge”, and a late sowing date are important management methods to combat other insect pests (Painter et al. 1954, Morrill and Kushnak 1999). Rather, knowing how landscape composition surrounding a field in the previous season affects the risk of Hessian fly infestation better enables producers to make informed management decisions. Further research is needed to verify if our results are consistent across the Great Plains region before our conclusion can be incorporated into Hessian fly IPM decisions, as we sampled only six fields within a relatively small area (two neighboring Kansas counties). Our study provides a starting point for future research, such as sampling Hessian fly distribution within fields spanning the extent of the Great Plains. Also, the effect of buffer size on Hessian fly distribution is untested, even though it is based on our current understanding of Hessian fly dispersal through the landscape (McColloch 1917, Withers et al. 1997). Future work could also focus on cultivars with improved resistance to Hessian fly, as there is a wide range in varietal responses (DeWolf et al. 2017) and genetic resistance to the pest exists in modern wheat genotypes (Edwards et al. 2012). Together, this research can lead to a better understanding of Hessian fly distribution in wheat fields and areas at higher risk for infestation; ultimately, aiding in the goal of improving Hessian fly IPM.

The last two decades have seen a growing interest in development of a monitoring technique that would better inform producers of Hessian fly populations to help make informed management decisions (Botha et al. 2005, Andersson et al. 2009, Schwarting et al. 2015).

Pheromone and light traps have been examined as potential monitoring techniques (Knutson et al. 2017, Schmid et al. 2017); however, the significant area dedicated to wheat production in the Great Plains necessitates that deployment of traps be refined to areas of higher risk of Hessian fly infestation. Tools categorizing landscape cover (e.g., Cropscape) with spatial resolution capable of classifying landscape within and surrounding fields, while also including a temporal scale relevant to the growing season are now readily accessible. Using these tools to identify fields surrounded by a small proportion of wheat within 1 km buffer could help focus Hessian fly monitoring efforts to fields located in a landscape matrix with higher potential for outbreaks.

Additionally, many other insect pests of the Great Plains migrate during the growing season, and landscape analysis could be important for monitoring these pests too. For example, the chinch bug, *Blissus leucopterus* (Say) (Hemiptera: Blissidae), immigrates throughout the growing season in Kansas from native grasses to wheat and other small grains, then to sorghum and corn, before returning to native grasses in the fall (Michaud and Whitworth 2013). Our results contribute to the growing study of landscape effects on pest populations, and how analysis of the landscape can help predict areas at higher risk for pest outbreaks (Margosian et al. 2009, O'Rourke et al. 2011, Mazzi and Dorn 2012, Tonnang et al. 2017). This study together with previous research of landscape effects on insect communities highlights the need to consider landscape effects on pest immigration through the landscape of the Great Plains. As the landscape of the Great Plains has become more simplified since large-scale cultivation of the prairies began, it is important to remember that landscape composition can affect the distribution of insect pests and also the implementation of management practices against those pests.

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Tables and Figures

Table 7.1. Description of pertinent agronomic parameters of six Kansas commercial wheat fields sampled for Hessian fly infestation. Variety Hessian fly resistance ratings ranges from 1 (highly tolerant) to 9 (highly susceptible). Field 1 was divided in two portions, where the south portion was planted to LCS Mint and central and northern portions were planted to Everest, while fields 5 and 6 were entirely planted to a blend of two varieties.

Field	Area (ha)	Variety (Hessian fly resistance)	Volunteer wheat control	Previous crop	Elevation at field center (meters)	Proportion grass within 1 km of field center	Proportion wheat within 1 km of field center
1	44.6	Everest (5), LCS Mint (9)	No	Maize	438	0.15	0.40
2	31.2	Everest (5)	No	Maize	436	0.21	0.11
3	21.6	Everest (5)	No	Maize	440	0.22	0.30
4	31.6	T158 (9)	Yes	Wheat	430	0.08	0.21
5	27.8	Blend of WB 4458 (9) and Armour (9)	Yes	Wheat	391	0.31	0.36
6	6.1	Blend of WB 4458 (9) and Armour (9)	Yes	Wheat	388	0.24	0.27

All fields had wheat and grass cover adjacent to a least one field edge, which was used to calculate the minimum distance of each point sampled to field edge, wheat cover, and grass cover. This information was included as a covariates in the generalized additive models (GAMs) along with other aspects of field history. Covariates included in the GAMs were:

- 1) minimum distance from sampled points to wheat
- 2) minimum distance from sampled points to grass
- 3) minimum distance from sampled points to field edge
- 4) elevation of sampled points
- 5) level of resistance of wheat variety
- 6) proportion of winter wheat within 1 km buffer of sampled points
- 7) proportion of grass within 1 km buffer of sampled points

Figure 7.1. Hessian fly infestation of six commercial wheat fields in Kansas. Each point within the fields represents the number of A) Hessian fly pupae and B) plants infested found in a meter row.

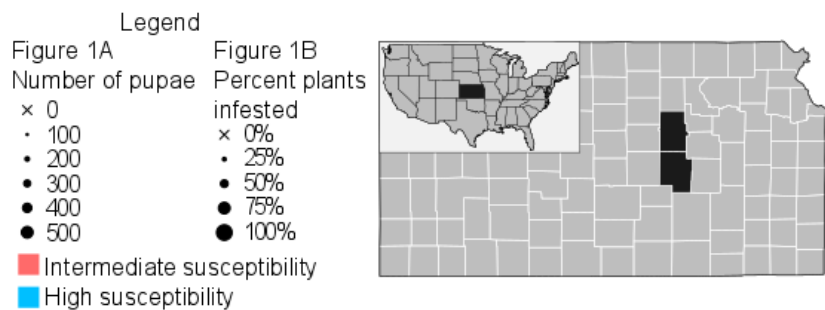
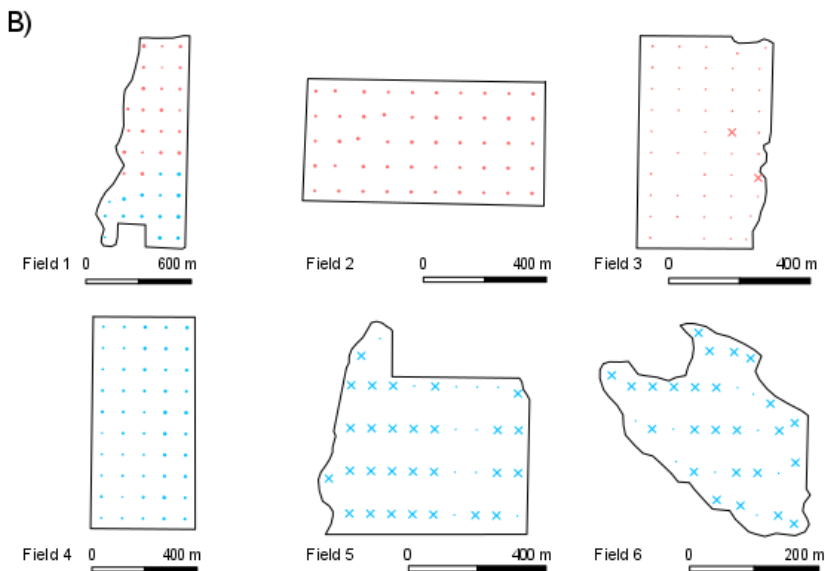
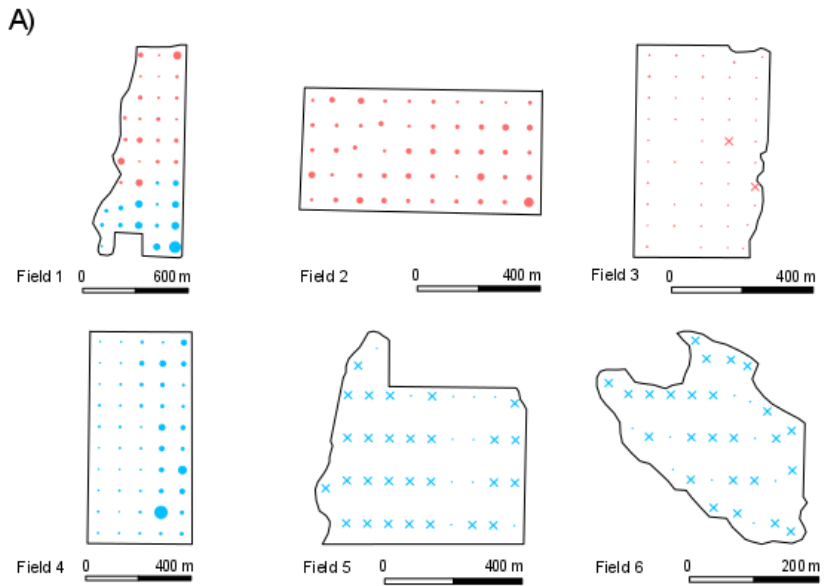
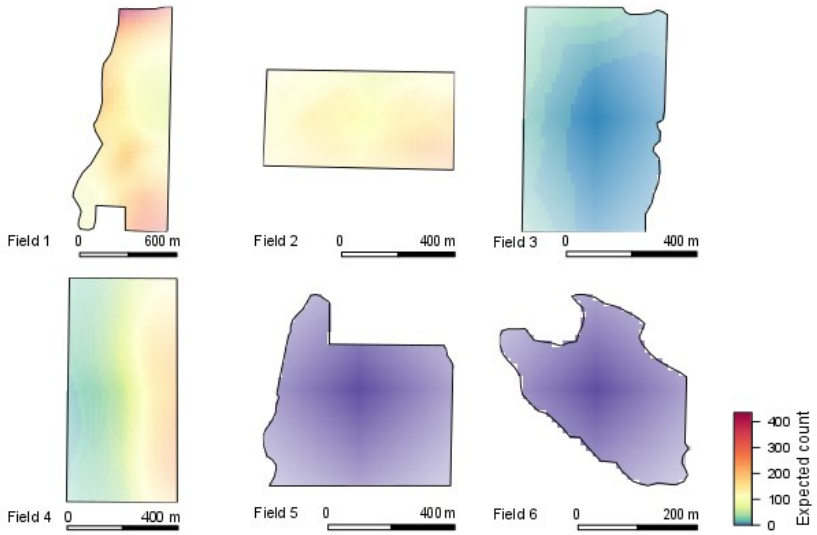


Figure 7.2. Predicted Hessian fly infestation of six commercial wheat fields in Kansas based on sampling data. A) Hessian fly pupae and B) plants infested within each field.

A)



B)

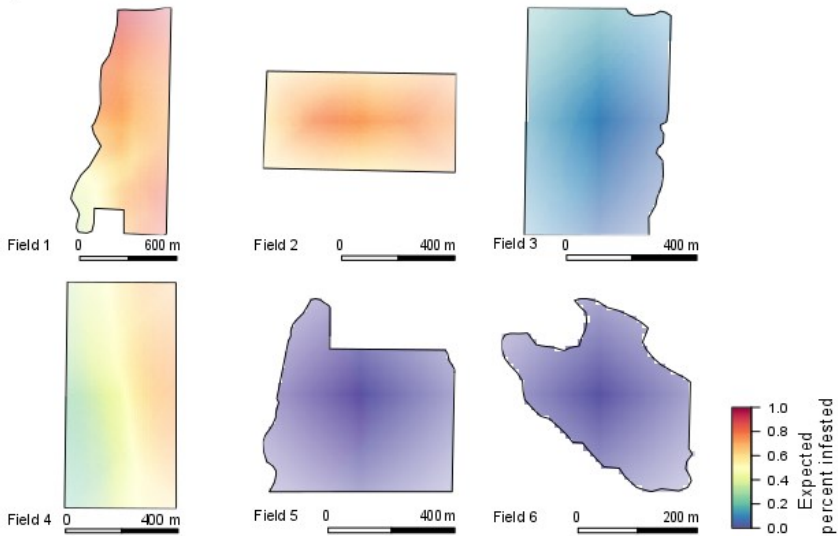


Figure 7.3. Maps of wheat cover in the previous growing season (highlighted in green) surrounding sampled fields (outlined in dashed yellow line).



Chapter 8 - Conclusion

This set of experiments has increased understanding of Hessian fly attraction to new sensor technologies (e.g., light emitting diodes (LEDs), and laser displays), and examined efficacy of these visual technologies in potential monitoring designs. Specifically, the experiments have shown the following about Hessian fly behavior:

- Attraction to wavelengths in the green spectrum (502 and 525 nm).
- Increased attraction to greater light intensity.
- The Hessian fly female sex-pheromone lure did not decrease female attraction to LEDs, and the lure increased male attraction to LEDs.
- Ambient light reduced attraction to LEDs.
- Wheat odor did not decrease attraction to LEDs.
- LEDs did not increase trap capture under field conditions.
- Green laser displays increased oviposition in wheat microcosms.
- Decreased proportion of wheat within a 1 km buffer surrounding a field increased distribution and probability of infestation between wheat fields.

Conclusions from the laboratory behavior experiments culminated in the demonstration that a green laser light display increased oviposition in wheat covered by the display. This shows the potential to work with naturally occurring environmental cues from wheat plants (i.e., odor and tactile cues) to concentrate Hessian fly oviposition; thus, increasing monitoring efficiency by providing a trap area with known dimensions that can be checked for Hessian fly infestations. Deployment efficiency of laser displays can be further increased by selection of fields with a higher risk of Hessian fly infestation, shown to be fields with a lower proportion of wheat in the 1 km surrounding landscape. This information can help to improve the currently available

Hessian fly monitoring method, (i.e., the female sex-pheromone trap) which captures male Hessian fly activity but does not correlate with infestation levels in the surrounding wheat field. The laser display could be used in conjunction with the pheromone lure trap, thereby monitoring for both male activity and increasing the monitoring efficiency of oviposition within the field. This would inform producers when adults are active in their fields, and advise that laser displays should be deployed to increase monitoring efficiency for larval infestations. The area covered by the laser display could then be managed according to the infestation level, with the goal to prevent dispersal to the rest of the field. Improved monitoring for Hessian fly could also benefit wheat producing countries or regions which have a vested interest to prevent Hessian fly incursion and dispersal (e.g., Australia). A monitoring strategy that can provide early detection (i.e., pheromone trap) of invasion coupled with a method to concentrate oviposition (i.e., green laser displays on wheat) could be incorporated into a mitigation protocol, as it can lead to quicker quarantine, prevention of dispersal, and eradication.

Together this work demonstrates Hessian fly behavior toward new technologies, and how the technologies interacts with previous Hessian fly trapping cues and environmental factors. However, it should be noted that not all flies deployed in the laboratory experiments responded to visual cues (LEDs). Even in the light arena bioassay examining effect of LED intensity on attraction where the wavelength of LEDs were fine-tuned (525 nm) to increase Hessian fly attraction, not all flies were attracted to the LEDs (51% and 90% of males and females were attracted to LEDs, respectively). This suggests that not every Hessian fly in the population (especially males) will respond to visual cues. Additionally, the Hessian fly population contains several biotypes which have overcome resistant genes in various wheat cultivars. As the Hessian flies used in these studies were all of the same biotypes, it would be important to study if the

difference in biotypes affects attraction to various wavelengths. Considering the response of flies to visual cues representing the entirety of the Hessian fly population would be an important next step for future research. Future research should also focus on the capability of laser displays to increase oviposition under field conditions, along with the optimum density of laser points required to increase oviposition. Additionally, monitoring methods for female Hessian flies should be further examined to develop a pre-planting detection technique that can inform implementation of integrated pest management (IPM) practices. For instance, deploying green laser displays on alternative hosts along the perimeter of fields at higher risk of infestation can increase producer awareness that female Hessian flies were active around a field prior to planting.

Ultimately, these studies have increased our knowledge of Hessian fly behavior toward new visual sensor technologies (LEDs and laser displays), and informed deployment of monitoring methods. Whether monitoring to inform IPM management in areas with established Hessian fly populations or monitoring to prevent incursion to countries absent of Hessian fly populations, the results of these studies demonstrate the potential to incorporate visual technologies with the existing pheromone trap to detect adult activity and concentrate oviposition to prevent dispersal. Together this information informs new methods to improve monitoring and management of the Hessian fly.

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Best regards,

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