CROP AND PRAIRIE GRASSES SERVING AS HOSTS FOR THE HESSIAN FLY *MAYETIOLA DESTRUCTOR* (SAY) (DIPTERA: CECIDOMYIIDAE)

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MASTER OF SCIENCE

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ABSTRACT

Insect herbivores typically parasitize a relatively small number of plant species. Host specialization is presumed to be a result of evolutionary arms races, with insect adaptations ultimately restricting host range. Being a gall-maker, the Hessian fly has highly evolved interactions with plant hosts. As a consequence, its host range is expected to be narrow. Two crop species, wheat and barley are hosts of the Hessian fly. I studied whether non-crop grasses can also serve as hosts. Included in tests were seven grass species that are important components of the grasslands of the Northern Great Plains. Although less suitable than wheat and barley, all seven species received eggs and five of the seven species supported development of offspring to the adult reproductive stage. Results indicate a broader host range than was expected. A benefit of being able to use non-crop grasses is availability of alternate hosts when superior crop hosts are not available.

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CHAPTER 1. LITERATURE REVIEW

Introduction

Insects and plants comprise a large proportion of all of the biological organisms that live on Planet Earth (Pimentel and Andow 1984, Andersson et al. 2009). It follows that the interactions that occur between insects and plants comprise a significant proportion of all of the planet's varied ecological interactions. These interactions can be antagonistic or mutually beneficial. The former include plants that must defend against herbivorous insects while the latter include plants that rely on insects for pollination services. The importance of relationships between insects and plants is evident in the evolutionary record where the radiation of insect species parallels the radiation of species of flowering plants (Ehrlich and Raven 1964).

Relative to insects, which are mobile and have highly evolved sensory and digestive systems, plants have few options for evading predators. Being hard to find is one option, but more commonly the plant evolves active defense traits (Jander and Howe 2008). There are various schemes for classifying these defense traits (Schoonhoven et al. 2005). One scheme distinguishes between direct defenses, in which plants develop traits to inhibit finding, feeding or digestion by insects, and indirect defense, in which the plant produces volatile signals that attract natural enemies, which then defend the plant. Another scheme divides defenses into traits that are in place all the time, i.e. constitutive defense, or defense traits that are triggered by attack, i.e. induced defense. A third scheme, commonly used by applied entomologists, places plant resistance traits into one of three categories (Painter 1951). Non-preference traits, which were subsequently renamed antixenosis (Kogan and Ortman 1978), allow the plant to avoid being attacked or to be attacked less than susceptible plants. Here the resistance trait prevents the insect from performing behaviors that are critical for colonization, for example, finding the plant or

feeding or laying eggs after the plant is found. Antibiosis traits negatively affect the survival, longevity, growth, and reproduction of the insect. For both nonpreference and antibiosis, we expect to see fewer insects on resistant plants then on susceptible plants. In contrast, tolerance traits improve the plant's chances of surviving attack. Here a resistant plant and a susceptible plant might be attacked by similar numbers of insects, but the resistant plant has a better chance of recovering from attack. Compensatory growth is a common tolerance trait, with this growth typically occurring after attack ceases. The defense traits that have been studied most intensively over the last 20 years are the chemical defenses that interfere with insect digestion or the behaviors that are necessary for colonization (Agrawal and Fishbein 2006, Jander and Howe 2008).

Scientists use their knowledge of plant defense mechanisms to protect the plant species that are used for food, fuel, and shelter (Wink 1988, Hammond-Kosack and Parker 2003, Bent and Mackey 2007). The first step is to find a plant resistance trait that has an economically significant impact on the pest. The second step is to determine the genetics underlying the resistance trait. The third step is to figure out if the resistance trait imposes a cost on the plant, with a cost expressed as a loss in crop yield or quality. The fourth step is to find a way to transfer the resistance trait to an elite crop cultivar. The final step is to deploy the resistance trait in a manner that ensures its durability, i.e. that it is effective over the long term. Unfortunately a tradeoff can occur between the durability of the resistance trait and the ease of moving it into elite cultivars. Single gene resistance traits are easiest to move but are viewed as the traits that are most vulnerable to 'defeat' by pest adaptation (Bent and Mackey 2007).

Resistant cultivars play an important role in integrated pest management today (Pedigo and Rice 2006) and will play an even greater role in the future when advances in genetics and

molecular biology make it easier to isolate plant resistance traits from 'alien' species and move the trait to an elite crop cultivar, e.g. from the wild grass *Aegilops tauschii* to domesticated wheat *Triticum aestivum* (Wang et al. 2006). An extreme form of this is moving a gene from something that is not a plant, e.g. the endotoxin gene from *Bacillus thuringiensis* (Bt), to a crop plant such as cotton or corn. Bt-protected crops were first deployed in the mid 1990s and today are grown on 68 million hectares worldwide (Huesing and English 2004).

To find novel plant resistance traits in 'alien' plant species, a first step is to define the host range of the pest species (Schoonhoven et al. 2005). This is necessary because we expect to find highly effective resistance traits at the interface of the set of plants serving as hosts and the plants that do not serve as hosts. Here an important distinction is made between host and non-host resistance (Heath 2000). If all of the genotypes within a particular plant species have defense traits that preclude insect attack, the plant species is considered to be outside the insect's host range, i.e. the plant is a non-host. This is non-host resistance. On the other hand, if only some of the genotypes within a particular plant species have defense traits that preclude attack, this plant species is considered to be a host. Genotypes within the species that do not serve as hosts are considered to have host resistance. Theoretically, non-host resistance is more durable or stable over evolutionary time, and therefore is more valuable for plant breeding programs (Heath 2000, Bent and Mackey 2007). Tolerance also is seen as more durable than antibiosis or nonpreference. This is because a plant that tolerates attack and recovers growth after attack puts less selection pressure on the pest than a defense trait that poisons the pest or prevents important reproductive behaviors (Schoonhoven et al. 2005).

The aim of my research was to explore the interactions between prairie grasses and the Hessian fly *Mayetiola destructor* Say (Diptera: Cecidomyiidae), an important economic pest of

crop species in the Tribe Triticeae (Berzonsky et al. 2003, Harris et al. 2003), especially wheat and barley. The Hessian fly is controlled almost entirely through the deployment of resistant wheat varieties. Thirty-two resistance genes, referred to as *H* genes (for Hessian fly resistance) have been identified. While this sounds like a sufficient number of resistance genes to control pest populations, the Hessian fly has proven itself capable of adapting to singly-deployed *H* genes, sometimes as quickly as two years after deployment of the *H* gene (Foster et al. 1991a, Gould 1998). Thus the search is on for novel resistance traits in wild grass species such as *Aegilops tauschii* (Yu et al. 2009). Scientists also are interested in finding new strategies for delaying or eliminating Hessian fly adaptation. Breeding more than one *H* gene into a crop, a process known as stacking or pyramiding, is expected to create more durable resistance to the Hessian fly (Porter et al. 2010). Right now this is not possible because we do not have the molecular markers that are necessary for stacking *H* genes.

Offensive Traits of Phytophagous Insects

A review titled "Herbivore Offense" (Karban and Agrawal 2002) describe the traits that allow insect herbivores to optimally exploit plants as hosts. Offensive tactics include the ability of insects to: make sophisticated choices for feeding or oviposition, produce salivary enzymes that minimize the effectiveness of plant chemical defenses, sequester biologically active host plant chemicals into tissues or glands to gain protection from predators and parasites, harbor bacterial symbionts in the gut to improve nutrients harvested from plants, and induce plant susceptibility by trenching behavior, gregarious feeding or the creation of plant galls. I will describe galls in further detail because of their relevance to the Hessian fly.

Galls are defined as aberrant plant cells, tissues, or organs that are stimulated by attack from foreign organisms (Redfern and Askew 1992). Organisms that induce galls in plants

include: viruses, fungi, bacteria, mites, nematodes and insects. An important group of insect gall-makers are the gall midges belonging to the family Cecidomyiidae (Gagné and Hatchett 1989). The newly eclosed gall midge larva induces gall nutritive cells at specific sites, commonly sites where cells are still young and expanding, i.e. cells that lack a mature wall. Protein synthesis and carbohydrate transportation are enhanced in the gall nutritive cells, with this providing the larva with a diet rich in soluble amino acids and sugars (Shorthouse and Rohfritsch 1992). Plant growth suffers as a result because photoassimilates are diverted from processes necessary for plant growth to processes necessary to create and maintain the gall maker's nutritive tissue. The plant can recover its growth after the gall maker completes the feeding stage.

Host Finding and Selection by Phytophagous Insects

Many holometabolous insect species feed as adults on nectar or pollen and do not feed on the plant that their offspring feed upon (Bernays and Chapman 1994). This means that the egg-laying adult female must choose a plant without having direct knowledge of the plant's suitability for larval feeding. Instead the female's choice is based on her ability to find the plant and her interactions with the external features of the plant, that is, the chemical and physical features of the plant surface. The responsibility of the adult female is particularly onerous if her newly eclosed offspring are unable to move to another plant or have limited energy stores and therefore cannot survive an extensive search of the plant they have been placed on by the adult female. The major holometabolous insect orders that contain plant-feeders are Coleoptera (beetles), Diptera (flies), Hymenoptera (sawflies), and Lepidoptera (butterflies and moths).

How do adult females select plants that are suitable for their offspring? Being critical for reproductive fitness, host-finding behavior is generally considered to be 'programmed behavior', that is, a predictable sequence of behavioral acts also known as a reaction chain (Schoonhoven et

al. 2005). A first step may be finding the habitat where the host plant commonly grows, cues here being green leaf volatiles and light and moisture levels. Having found this habitat, the insect perceives the plant at a distance, via plant-derived optical and/or olfactory cues, and does this while flying, walking, or resting (Bell 1990, Bernays and Chapman 1994). The insect then moves towards the plant and, upon making contact, proceeds to examine the plant by bringing various sensory systems, including chemoreceptors and mechanoreceptors, in contact with features of the plant surface (Visser 1988, Schoonhoven et al. 2005). These features include chemicals in the epicuticular waxes that cover the leaf surface (Barthlott et al. 1998), ridges and cavities associated with venation and stomata (Juniper and Southwood 1986), hairs and trichomes (Chiang and Norris 1983, Juniper and Southwood 1986, Werker 2000), and signs of occupation by other insects, e.g. frass, leaf damage, and eggs (Schoonhoven et al. 2005). Insects can also assess the overall structure of the plant, including its height, diameter and shape, by running over plant surfaces (Clark and Messina 1998, Cloyd and Sadof 2000). This is referred to as the kinesthetic sense because complex information is gained from assessing patterns of movement. After analyzing the chemical and physical characteristics of the plant, the insect decides to either lay one or more eggs or depart without laying eggs (Schoonhoven et al. 2005). After examining the plant and laying a few eggs, the female sometimes repeats the sequence of behaviors to examine the plant and then again choose to lay eggs or depart.

Does Mother Know Best? Preference and Performance

Optimal oviposition theory is an important approach for studying host selection by insects (Scheirs and De Bruyn 2002), and is also referred to as the preference-performance hypothesis (Jaenike 1978). Both assume that adult females are able to maximize their fitness by ovipositing on (preferring) the hosts that provide the best growth and survival (performance) for

their offspring. Many studies have reported that “mother knows best”, that is, their data showed positive correlations between adult preference and offspring performance (Levins and MacArthur 1969, Jaenike 1978, Mangel 1987, Ward 1987). Nevertheless, other studies have reported that “mother doesn’t always know best”, that is, data showed poor correlations between adult preference and offspring performance (Thompson 1988, Jaenike 1990, Thompson and Pellmyr 1991).

To explain poor correlations between adult oviposition preference and offspring performance, researchers have taken various approaches. Some explain the discrepancy by showing the importance of top-down forces (Bernays and Graham 1988, Dyer 1995, Berdegue et al. 1996, Bjorkman et al. 1997, Camara 1997, Gratton and Welter 1999, Stamp 2001). For example, some insects prefer a mediocre host that provides enemy-free space rather that a better host that threatens the survival of offspring because it is more likely to be visited by predators. Other scientists have claimed that the physiological state of the adult female is important (Minkenberg et al. 1992). For example, the egg load (i.e. the number of mature eggs in the ovaries) carried by the insect generates variation in host choice: given the same life span, an adult female that carries 400 eggs will be more likely to accept a suboptimal host than a female that carries only 20 eggs. There can also be constraints on information processing that lead to poor choices (Bernays 2001). For example, generalist insects that must choose among hundreds of possible host species have a greater sensory challenge than specialist insects that only choose between 2-3 species. Limitations in learning processes can also create constraints on host choice (Dukas and Bernays 2000, Egas and Sabelis 2001). The adult female’s need to find food for herself also can compromise the search for food for her offspring (Scheirs and De Bruyn 2002). Thus, there can be a trade-off between what is good for the mother and what is good for her

individual offspring. For example, to feed herself the female may need to stay near nectar sources, but the best plants for her offspring may be elsewhere. Finally, if the association between the plant and insect is new, the insect may not have had enough time to evolve the capacity to discriminate between optimal and less optimal hosts (Thompson and Pellmyr 1991).

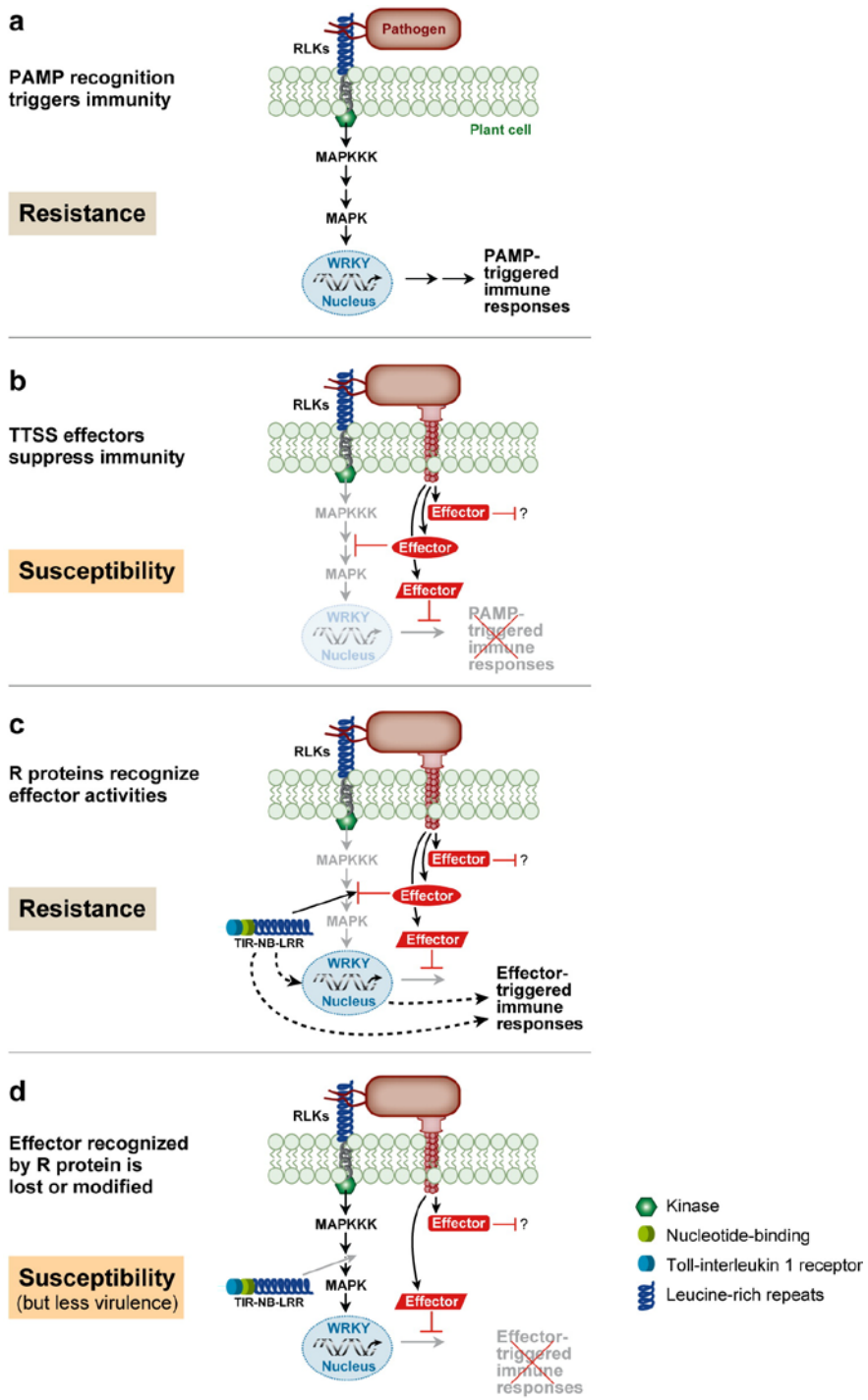
Gene-for-Gene Plant Resistance

Harold Henry Flor (1956) proposed the gene-for-gene concept to explain the interaction between the *resistance* (*R*) gene of a plant, flax *Linum usitatissimum*, and the *avirulence* (*Avr*) gene of a pathogen, flax rust *Melampsora lini*. In this model, the dominant *R* gene of the plant can provide resistance for the plant if the pathogen expresses the corresponding dominant *Avr* gene. The model was proposed to explain the patterns of plant resistance and pathogen virulence that Flor observed in the field, where a single *R* gene was deployed in a flax cultivar and conferred resistance to flax rust for a number of years but then eventually lost its effectiveness.

The biochemical basis of gene-for-gene interaction has been an important research topic over the last twenty years (Bent and Mackey 2007). The "elicitor-receptor" model has gained acceptance since the gene-for-gene concept was proposed (Keen 1990, Staskawicz et al. 1995, Bent and Mackey 2007). In the 'elicitor-receptor' model, a specific receptor encoded by the *R* gene directly recognizes and interacts with the product encoded by the corresponding *Avr* gene. This pathogen product triggers a defense response in the plant and therefore is seen as an 'elicitor' of plant defense. This defense commonly results in the parasite being harmed or even killed. If the plant lacks the *R* allele, there is no 'receptor' for the *Avr* product and no defense response is triggered. As a result the parasite is able to colonize the plant and survives and grows, the result being damage to the plant. If the parasite does not produce the *Avr* product, for example because of an *Avr* mutation, no 'elicitor' is produced and plant defense is not triggered.

A recent paradigm shift in plant pathology has led to a more complete model of *R/Avr* interactions (Hogenhout et al. 2009), which is nicely summarized in the four-part model presented in a review by Bent and Mackay (2007) (Figure 1). The most important difference between this model and previous models is the recognition that parasite *Avr* products have two functions. The first function is for the parasite, which uses the *Avr* product during colonization to suppress defense or to change plant physiology to benefit parasite growth, an example here being the creation of a specialized feeding tissue like a gall. Because the *Avr* product benefits the parasite, here the *Avr* product it is referred to as an 'effector'. The second function comes when the plant has a matching *R* product that allows the *Avr* product to be used as a means for detecting attack. Here because the *Avr* product benefits the plant with the matching *R* gene, the *Avr* product it is referred to as an 'elicitor' of plant defense. Because of this dual 'effector/elicitor' function, Avr proteins are said to have two faces, like the Roman god Janus (Bent and Mackey 2007, Hogenhout et al. 2009).

The model of Bent and Mackey (2007) (Figure 1) includes four parts for the evolutionary process of plant-pathogen interaction: first, the plant recognizes 'pathogen associated molecular patterns' (PAMP) and triggers immunity; second, some parasites evolve effectors (Avr effector) that are secreted by various systems (e.g. the type three secretion system, TTSS, (Salmond and Reeves 1993)) to suppress immunity, and finally become adapted to the plant defense; third, some plants evolve to be able to produce the R protein to detect (either directly or indirectly) the Avr effector when exposed to those adapted parasites; and fourth, a certain portion of parasites, again, become adapted to the plant's *R* gene-mediated detection by *Avr* gene loss of function. In the first part of the model (Figure 1a), the plant recognizes attack by the pathogen: signals generated by the attack are transmitted to the nucleus where DNA transcription is adjusted to



Bent AF, Mackey D. 2007.
Annu. Rev. Phytopathol. 45:399–436

Figure 1. Four-part *R/avr* interaction model (from Bent and Mackay 2007).

produce defense responses. This stops further attack by the pathogen. In the second part (Figure 1b), the adapted pathogen injects an Avr effector, which suppresses plant defense. Now the parasite improves its chances of survival and growth. In the third part (Figure 1c), the plant evolves and now has a matching R product, which interacts with the Avr effector generated by pathogen and triggers immune responses to prevent further damage from the pathogen. In the last part of the model (Figure 1d), the loss of *Avr* gene function of certain parasite means that the plant with the matching *R* gene no longer detects parasite attack. Now the parasite has "defeated" *R* gene-mediated resistance. In this four-part model, the interactions between insect offense and plant defense are like an arms race, both sides evolving to gain the upper hand. Since we, as agriculturalists, are on the side of the plant, the parasite's ability to adapt creates an important challenge for scientists who seek durable plant resistance (Chen et al. 2009b).

Non-Host Resistance

If all of the genotypes within a particular plant species have defense traits that preclude insect attack, the plant species is considered to be outside the insect's host range, i.e. the plant is a non-host. In contrast, if the plant species contains some genotypes that allow survival, growth, and reproduction, and other genotypes that have defense traits that preclude attack, the plant species is a host and the genotypes exhibiting resistance are said to have host resistance. Theoretically non-host resistance should be more durable than host resistance. Non-host resistance is the resistance exhibited when all genotypes within the species are resistant to the parasite (Heath 1997, Mysore and Ryu 2004). Non-host resistance is presumably more complicated than host resistance, relying on multiple defense mechanisms including: constitutive barriers that involve physical defenses (e.g. thicker cell wall) and chemical materials (e.g. alkaloids), and induced responses at attack sites, which include formation of papillae to reinforce

the cell wall, and the hypersensitive response, which includes cell death (Wolter et al. 1993, Kamoun 2001, Thordal-Christensen 2003, Trujillo et al. 2004, Nurnberger and Lipka 2005).

Geographic Distribution of the Hessian Fly

The Hessian fly is believed to be a native of Southwest Asia, which is the center of origin of wheat. It is now found in North America, southern Europe, Asia, North Africa, and New Zealand (Barnes 1956, Gagné and Hatchett 1989, Pauly 2002, Harris et al. 2003). In North America, the Hessian fly occurs from the Atlantic Coast to the Great Plains, and also occurs in some areas of the Western United States, including portions of California, Oregon, Washington, and Idaho (Ratcliffe et al. 2000).

Systematics of Gall Midges and the Hessian Fly

The Hessian fly belongs to the family Cecidomyiidae within the order Diptera. Cecidomyiids are a particularly interesting family in evolutionary terms due to their rapid rate of species formation compared with their close relatives in the Bibiomorpha (Mamaev 1975). Many cecidomyiid species feed on fungi or decaying plants but the cecidomyiids that feed on living plants are characterized by their capacity for making galls. The Hessian fly belongs to the tribe Oligotrophini, one of three tribes that contain gall makers. *Mayetiola* is efficient at breaking down host tissues before ingestion, with well-developed salivary glands and a larval intestine with an enlarged midgut (Mamaev 1975). Another character of *Mayetiola* is the lack of feeding by the third instar larva, which is really a pre-pupal stage within the shed skin of the second instar larva. Aestivation and diapause can occur during the third instar phase.

Morphology of the Hessian Fly

The adult fly is a small, long-legged, two-winged insect that resembles a mosquito. The female fly, about 4 mm long, has a distinct reddish tinge. The male is darker and has a smaller

body, but longer legs and wings. The wing length of the Hessian fly is related to its reproductive potential (Bergh et al. 1990, Withers et al. 1997, Harris et al. 2001). For the female, the wing length is correlated positively with the number of eggs in the ovaries. For the male, wing length is correlated positively with the number of females that can be inseminated (Barnes 1956, Bergh et al. 1990, Withers et al. 1997). Eggs are red and oblong, and they are deposited individually on the upper surface of wheat leaves. Under 10X magnification, eggs can clearly be seen lying in parallel grooves of the upper leaf surface. The newly hatched larvae are also red for 2-3 days before turning white. As larvae mature, a translucent green stripe appears down the middle of the back, which represents the plant juices that fill the digestive tract. The maggot is about 5 mm long when full grown. The maggot transforms into an adult fly inside a dark brown case, or puparium, that resembles a flaxseed in size and shape. Newly formed puparia are a lighter brown color that transforms, over time, to a mahogany brown color with age. Puparia or "flaxseeds" are located under leaf-sheaths and usually below ground on young tillers, or below the joint in older plants.

Life Cycle of the Hessian Fly

The life span of the adult Hessian fly is very short, usually less than three days (Enoch 1891, Bergh et al. 1990, Harris and Rose 1991). The majority of male Hessian flies emerge in the late afternoon, 12 hours earlier than females, but these males do not become active until approximately 10 hours after emergence (Bergh et al. 1990). Females emerge in the early morning and immediately begin releasing a volatile sex pheromone (Anderson et al. 2009), which the males use for orientation (Bergh et al. 1990, Harris and Foster 1991). Mating commences and is completed by late morning. Females mate only once, while males mate up to 30 times (Stokes 1957, Bergh et al. 1992). After mating, the female is quiescent for 1 – 3 hours

and then becomes active, beginning the search for egglaying sites on the leaf blade of hosts. It takes three to five days for the egg to hatch (McColloch and Salmon 1923). After hatching, the larva turns its body 180° and crawls down the leaf to the base of the plant, attacking the abaxial surface of a young, still-expanding leaf (McColloch and Yuasa 1917). On older plants, the attack site is just above the point of attachment of the leaf sheath to the stem. At attack sites the larva attacks epidermal cells and feeds on cell contents. The larva has three developmental stages: the small red larva, then a white maggot up to 5 mm, followed by a non-feeding larval stage inside the loosened larval skin (puparium) (Gagné and Hatchett 1989). The larval stage lasts for 16 to 25 days or longer if in diapause (Gagné and Hatchett 1989). The pupal period lasts six to 33 days depending on temperature and humidity (Walkden 1936). The Hessian fly has two to four generations per year in different region, for example, in North Dakota, it has two generations, while in Georgia, it has four generations per year.

Host Finding and Selection Behavior of the Hessian Fly

Oviposition decisions are important because they help determine host range (Wiklund 1975). Chemical, visual, and physical features of the wheat plant stimulate oviposition (Harris and Rose 1989, Foster et al. 1991c, Foster et al. 1991b, Harris et al. 1993, Kanno and Harris 2000a, Morris et al. 2000). Hessian fly uses visual cues to distinguish between grasses and non-grasses (Harris et al. 1993). While the Hessian fly female hovers in flight close to the grass leaf, volatile chemicals and leaf color stimulate its landing (Foster and Harris 1992, Harris et al. 1993). After landing, it arches its body so that the tip of the abdomen touches the leaf surface. Then it moves its antenna to sense leaf chemicals and moves the tip of its abdomen across the leaf at right angles to detect features associated with leaf veins. The physical features of the leaves influence the female's egg-laying decision. The female prefers to lay eggs on the adaxial

leaf surfaces of wheat, which is characterized by ribs and by the grooves that lie between the ribs (Kanno and Harris 2002). Both physical features, i.e. grooves, and chemicals, i.e., 1-octacosanal and 6-methoxy-2-benzoxazolinone (MBOA) (Morris et al. 2000) stimulate egg laying. After ovipositing eggs, the female sits for only short periods before again taking flight.

Hessian fly females sometimes choose plants that do not support the development of their offspring (Harris et al. 2001). The Hessian fly adults only live for three days at most and have only 2-5 hours to find plants for their 200-450 eggs, laying only 1-2 eggs on each plant they encounter (Enoch 1891, Bergh et al. 1990, Harris and Rose 1991). Thus, they may not have enough time to find the best hosts. Another possibility is that the presence of the *H* gene in the wheat plant, which confers resistance, leaves no sign on the leaf surface that can be detected by the ovipositing female (Harris et al. 2001).

The Hessian fly as a Gall-maker

Most galls consist of nutritive tissue, i.e. the cells that provide food for the larva, and the outgrowth that surrounds the nutritive tissue. Since the gall outgrowth is the only part of the gall that is visible to the human eye, the focus has been on this outgrowth, including its fantastic variety of shapes and colors. However, it is the nutritive tissue that has the greatest benefit for the larva. It has been asserted that the gall outgrowth also benefits the larvae by providing a shelter from the elements, as well as predators and parasites (Hutchins 1969). But data do not support this. Indeed the gall outgrowth appears to make the gall maker more visible to some predators, such as birds (Weis and Abrahamson 1985).

The Hessian fly induces serious growth deficits in the seedling plant but does not induce a visible gall outgrowth (Barnes 1956). Because of this, the Hessian fly had been seen as an anomaly among cecidomyiid plant-feeders, that is, a gall midge that feeds in some way that does

not involve creation of a gall. However, recently it was discovered that the Hessian fly is a gall-maker, producing nutritive tissue (Harris et al. 2006b) that is similar in all respects to the nutritive tissue produced by other gall-makers, including cecidomyiids and cynipid wasps (Shorthouse and Rohfritsch 1992). For the Hessian fly, the process of creating a nutritive tissue occurs as follows (Harris et al. 2006b). After hatching from the egg, which is deposited by the adult female on the adaxial surface of the blade of the youngest leaf of the wheat seedling (Harris and Rose 1989), the larva crawls down the leaf blade to the base of the sheath, which is enclosed within the sheathes of older leaves. The larva then moves to find the abaxial surface of the adjacent younger leaf and uses its specialized paired mandibles to attack epidermal cells found in the zone of cell elongation. Mandibles of the first instar are bladelike, tapering distally to a single, sharp-pointed tooth, which is adapted for making shallow holes in the plant cell wall (Hatchett et al. 1990, Harris et al. 2006b, Harris et al. 2010). Ducts from each of the paired salivary glands lead to the paired mandibles and allows secreted salivary gland fluids to be injected into the plant cell wall (Hatchett et al. 1990, Harris et al. 2010).

In the susceptible plant, this physical and chemical attack has two results. First, it causes the wall of attacked cells to break down, releasing partially digested cell contents to the leaf surface where the larva applies its head and vacuums up its liquid diet (Harris et al. 2006b). The second thing that happens is that epidermal and mesophyll cells at attack sites develop into nutritive cells. After being created, these nutritive cells, like the non-nutritive cells that the Hessian fly larva first feeds on, break down their walls, again delivering liquid food to the larva. This food from nutritive cells is enriched in amino acids and sugars (Liu et al. 2007, Zhu et al. 2008). From this point on the larva grows rapidly (Gagné et al. 1991). Studies using stains to

show changes in cell permeability show that the entire attack zone on the leaf sheath ends up leaking nutrients to sessile Hessian fly larvae (Williams et al. 2011).

Hessian fly Impacts on Plant Growth

The impacts of Hessian fly attack on the growth of susceptible and resistant wheat seedling have been quantified (Anderson and Harris 2006, 2008). If wheat seedlings are infested in the two-leaf stage, the third leaf shows how quickly responses of susceptible and resistant plants diverge (Anderson and Harris 2008). For the third leaf, larvae have an effect on the growth of both plant resistant and susceptible plant types, but susceptible plants suffer a much greater loss (Anderson and Harris 2008). Growth impacts on the fourth leaf shows that negative effects of larval attack extend beyond the third leaf. However, whereas the fourth leaf of susceptible plants either grows very little or does not grow at all, the fourth leaf of resistant plants shows only minor growth deficits of 1-2 cm.

It is interesting that both susceptible and resistant genotypes showed negative effects in the fourth leaf. Larvae use their mandibles to puncture the outer cell wall of epidermal cells on the abaxial side of the third leaf on both susceptible and resistant genotypes (Harris et al. 2006b, Harris et al. 2010). Even though nutritive tissues cannot be triggered on resistant plants, there may be a cost for the induced resistance responses of resistant plants (Anderson and Harris 2008).

The Hessian fly and Gene-for-gene Interactions

The Hessian fly is one of a handful of insects that shows gene-for-gene interactions with host resistance *R* genes (Harris et al. 2003). In field deployment of single *H* genes (*H* gene being the name of *R* genes that confer resistance to the Hessian fly), we see the same pattern of success as was seen with Flor's flax-rust system: a single wheat resistance gene, e.g. *H3* or *H6*, provided

resistance to Hessian fly populations for 3-8 years and then was defeated by Hessian fly adaptation, which presumably occurred through selection, via the *H* gene, of virulent individuals within the population (Foster et al. 1991a, Gould 1998). In spite of the ability of the Hessian fly to adapt, plant resistance is considered to be the most economical and environmentally sound method of controlling Hessian fly populations (El Bouhssini et al. 2001, Berzonsky et al. 2003, Porter et al. 2010).

The Hessian fly is similar to plant pathogens in eliciting cell wall fortification in resistant plants (Harris et al. 2010). While cells directly attacked by the larva's mandibles exhibit the hypersensitive response (i.e. programmed cell death), adjacent epidermal and mesophyll cells show an accumulation of endoplasmic reticulum, numerous small vesicles associated with the Golgi bodies, an increase in the surface area of the plasma membrane, a separation between the plasma membrane and the cell wall, and a thickening of the outer cell wall (Harris et al. 2010). These induced defenses presumably prevent the attacking larva from creating nutritive cells and breaking down the cell wall (Harris et al. 2010, Anderson et al. 2011, Williams et al. 2011). The larva appears to starve to death, either because it fails to acquire life-sustaining nutrients or because it is poisoned and is unable to feed. In resistant plants, there is a transient period of cell wall permeability that may allow toxic lectins to reach the attacking larva (Williams et al. 2011).

Because of Hessian fly adaptation to resistance genes, the search continues for new *H* genes and new strategies for deploying *H* genes (Gould 1998). To date, 33 *H* genes or alleles have been identified from wheat or wheat relatives, including rye, *Secale cereale* L., and goat grasses, *Aegilops* spp. (Liu et al. 2005, Sardesai et al. 2005a). A study of the efficacy of *H* genes for controlling Hessian fly populations from the southeastern United States showed that only five out of the 21 genes, *H12, H18, H24, H25,* and *H26,* provided effective control (Cambron et al.

2010). This indicates that we need a better strategy. Simply continuing to identify *H* genes and inserting single genes into the wheat genome is time consuming and costly. Either we need better *H* genes that the Hessian fly cannot adapt to or we need to stack or pyramid multiple *H* genes to delay or prevent Hessian fly adaptation (Harris et al. 2003, Chen et al. 2009a, Porter et al. 2010).

Why Study Host Range of the Hessian Fly?

Understanding interactions between the Hessian fly and its grass hosts is important for a number of reasons. First, there is a fundamental question about the limitations that the gall-making habit places on host range. The generally accepted idea is that forcing a plant to make a gall limits the gall-maker to a small number of closely related plant species that share some essential feature that makes them vulnerable to this manipulation. Having a broader host range as a gall-maker, as well as the ability to expand host range, may signify that the gall-maker is manipulating a feature that is shared by a larger number of plant species. If this is the case, excluding the gall-maker may require an active defense, rather than a defense that consists of simply refusing to react, this being a well-cited hypothesis about how plants defend themselves against gall-makers (Shorthouse et al. 1992, Hutchins 1992, Miller 2004).

This brings us to the second reason why studying the host range of the Hessian fly is important. Non-crop grasses may be important sources of resistance to the Hessian fly, specifically providing resistance genes that can be transferred to crop cultivars and used alongside other resistance genes to develop durable resistance to the Hessian fly. Intermediate wheatgrass and tall wheatgrass are example of non-crop grasses that have provided traits that confer resistance to pathogens and pests (Cai et al. 1996, Xin et al. 2001, Sibikeeva et al. 2004, Ayala-Navarrete et al. 2007).

A third reason for studying the host range of the Hessian fly is to better understand the population dynamics and genetics of the Hessian fly, including a better understanding of where virulence to *H* genes evolves. In theory, virulence to *H* genes evolves in crop fields where *H* genes are deployed in resistance cultivars (Foster et al. 1991a, Zantoko and Shukle 1997). However, this theory is contradicted by many studies (Naber et al. 2000, Ratcliffe et al. 2000, Naber et al. 2003) showing virulence to specific *H* genes in Hessian fly populations that have never knowingly been exposed to the *H* gene. From this observation, and similar observations in other pests (e.g. the greenbug aphid) that attack wheat as well as wild grasses (Porter 1997), has come a different idea that is now gaining traction among entomologists and plant pathologists. This is that genetically-based virulence to resistance genes evolves during the time that pests spend outside of crop fields, that is during their exposure to wild and pasture hosts that also contain resistance genes (Porter et al. 1997).

A final reason for studying interactions between the Hessian fly and the non-crop grasses in Table 1 is that these grasses are an important component of grasslands, which themselves are important because they provide food and shelter for animals, including farm animals, birds, and small mammals. Non-crop grasses also are being examined as possible sources of cellulosic ethanol, an example being tall wheatgrass *Thinopyrum ponticum* Podp. (Zheng et al. 2007).

The Crop Hosts of the Hessian Fly

Wheat is the Hessian fly's best-known host. It is an economically important grain crop in the world due to its high nutrient and yield (Chapman and Peat 1992). The production of wheat, which was 680 tons in 2009 in the world, ranks second after corn. Even though the production of wheat keeps growing, there is still a strong desire for increasing the yield of wheat because there are still more than 925 million people in the world suffering from chronic hunger (FAO 2010).

The yield of wheat is mainly constrained by biotic stress, including stress from insects, pathogens, nematodes and weeds, and by abiotic factors, including stress from drought, salinity, and frost. The main idea of reducing or even solving these problems is to develop more effective and durable resistant cultivars that are resistant to biotic and abiotic stress.

Understanding the evolution of wheat is important for finding resistance genes. Wheat is considered to have originated from the Near East, such as Syria, Jordan, and Turkey (Lev-Yadun 2000). Based on numbers of chromosomes, wheat is categorized as diploid, tetraploid, and hexaploid. Einkorn wheat is a diploid species that has two sets of chromosomes, AA. Durum wheat is a tetraploid wheat with two diploid genomes that are denoted as AA and BB. Bread wheat is a hexaploid species with three diploid genomes that are named AA, BB, and DD, originated from three different species. For tetraploid wheat and hexaploid wheat, species from *Aegilops* and *Triticum* are the donors of their B genomes and D genomes (Nishikava 1980, Wang et al. 1997). Therefore, the *R* genes found in *Aegilops* and *Triticum* are easy to transfer to wheat.

Barley is the Hessian fly's second best-known host. Barley is also an economically important crop with a production ranking of 4th among cereals in 2005 (FAOSTAT 2005). It is widely planted in Germany, France, Ukraine, Russian, Spain, North America, North Africa and Australia (FAOSTAT 2010). Barley is used to produce flour, beer, distilled alcohol, barley tea, syrup, and animal food. The origin of barley is considered to be the Near East or Tibet, but is still under discussion. Barley is a diploid species with 2n = 14 chromosomes. It is more adapted to tolerate soil salinity than wheat, but less adapted to low temperatures than winter wheat. The major diseases of barley are leaf spot *Erwinia carotovora* subsp., rust *Puccinia triticina*, and powdery mildew *Phyllactina* spp; the major pests of barley are Russian wheat aphid *Diuraphis*

noxia; Hessian fly can also severely damage barley (USDA-NRCS 2012). The best way to control barley disease and pests is to use resistant cultivars (USDA-NRCS 2012).

Grassland Ecosystems

White et al. (2000) defined grasslands as "terrestrial ecosystems dominated by herbaceous and shrub vegetation, and maintained by fire, grazing, drought and/or freezing temperatures". Grasslands are an important resource in many aspects (Gibson 2009). First of all, they protect and conserve soil and water resources. Second, grasses serve as forage for livestock. Third, grasslands provide food and habitat for wildlife. Fourth, grasslands help increase biodiversity on the earth, providing habitat for thousands of plant species, but also insects and birds. Fifth, grasslands can also help store carbon by photosynthesis. Finally, in recent years, scientists have developed a new usage for grasses, this being biofuel.

Grasslands are distributed on all the continents except the Antarctic (Gibson 2009), covering approximately 40% of land area (White et al. 2000). Grasslands are the most wide-spread in sub-Saharan Africa, followed by Asia, Europe, North America, and Oceania (White et al. 2000).

In the United States, grasslands cover the Northern Great Plains, which are also defined as prairie (Forage and Grazing Terminology Committee 1992). Table 1 shows 22 common warm season and cool season grasses in the Northern Great Plains. Some of them are native grasses, e.g., meadow brome, smooth brome and Canada wildrye; and some of them are introduced, e.g. intermediate wheatgrass, and tall wheatgrass. Most of the introduced grasses originated from Eurasia. Among these grasses, tall wheatgrass, intermediate wheatgrass, slender wheatgrass, crested wheatgrass, smooth brome, and western wheatgrass are the most common grasses in North Dakota.

Table 1. Common grasses in the Northern Great Plains. The most common grasses in North Dakota are in bold (from Kevin Sedivec, NDSU).

Scientific Name	Common Name
Cool season grasses	
Agropyron cristatum	**Crested wheatgrass**
Bromus inermis	**Smooth brome**
Bromus riparius	Meadow bromegrass
Elymus canadensis	Canada wildrye
Elymus trachycaulus	**Slender wheatgrass**
Leymus angustus	Altai wildrye
Leymus cinereus	Basin wildrye
Nassella viridula	Green needlegrass
Pascopyrum smithii	**Western wheatgrass**
Psathyrostachys juncea	Russian wildrye
Pseudoroegneria spicata	Bluebunch wheatgrass
Thinopyrum intermedium	**Intermediate wheatgrass**
Thinopyrum ponticum	**Tall wheatgrass**
Warm season grasses	
Andropogon gerardii	Big bluestem
Andropogon hallii	Sand bluestem
Bouteloua curtipendula	Sideoats grama
Bouteloua gracilis	Blue grama
Calamovilfa longifolia	Prairie sandreed
Schizachyrium scoparium	Little bluestem
Sorghastrum nutans	Indiangrass
Panicum virgatum	Switchgrass

Prairie Grass Species Used in My Research

Canada Wildrye

Canada wildrye *Elymus canadensis* L. is a native cool season short-lived perennial grass distributed throughout the northeastern, northern, and western United States (USDA-NRCS 2012). It is used for forage for livestock, food for wildlife, and erosion control. It mainly grows on sandy shores and wooded areas. It is adapted to drought, salinity, and shade (USDA-NRCS 2012). As the crown of Canada wildrye has coarse stems and leaves, it is resistant to fire mortality to a certain degree. Canada wildrye seedlings develop fast, but it is not a competitive grass. The major diseases of Canada wildrye are leaf and stem rust *Puccinia triticina*, and the major pests are fall armyworms *Spodoptera frugiperda*, and rice stink bugs *Oebalus pugnax* (USDA-NRCS 2012).

Crested Wheatgrass

Crested wheatgrass *Agropyron cristatum* L. is an introduced cool season long-lived perennial grass and is adapted to the western United States (USDA-NRCS 2012). It was introduced from eastern Europe and Asia to the Great Plains area of the United States in 1898 (USDA-NRCS 2012). It is used for forage production and, once established, can stand high grazing pressure (65% use or higher). Crested wheatgrass can also be used for building soils as it has a strong root system (USDA-NRCS 2012). Crested wheatgrass is adapted to a variety of soil conditions. It competes well with other grasses, which makes it an invasive grass and the native grasses cannot coexist with it (USDA-NRCS 2012). Few pathogens attack crested wheatgrass, while it is a major host of the black grass bug *Labops hesperius* (Hannaway and Larson 2004).

Intermediate Wheatgrass

Intermediate wheatgrass *Thinopyrum intermedium* Barkworth & D.R. Dewey is an introduced cool season long-lived perennial grass (USDA-NRCS 2012). It originated from Asia around the Black Sea. In the United States, intermediate wheatgrass is distributed in the Northern Great Plains, west to central Washington, and south into Colorado, Kansas, northern New Mexico, and Arizona (USDA-NRCS 2012). It is a hay grass with high yield. It serves as food for cattle, sheep, and horses, but needs to be managed carefully to allow continuous grazing (USDA-NRCS 2012). Intermediate wheatgrass is also used for erosion control and building soils due to its heavy root production. Some cultivars of intermediate wheatgrass, e.g. ‘Reliant’, have been used to develop resistant traits against pathogens (USDA-NRCS 2012). ‘Reliant’ intermediate wheatgrass developed by the North Great Plains Research Laboratory (Mandan, ND), is adapted to the Northern Great Plains region and used for forage and seed production. Intermediate

wheatgrass is not an invasive species because it spreads slowly. Very few individuals spread through seed distribution. It has been reported that tan spot *Pyrenophora tritici-repentis* is the major disease of intermediate wheatgrass, and the major pest of it are grasshoppers *Melanoplus differentialis* (Thomas) (USDA-NRCS 2012).

Tall Wheatgrass

Tall wheatgrass *Thinopyrum ponticum* Podp. is an introduced cool season long-lived perennial grass (USDA-NRCS 2012). It originated from western Asia, i.e., Turkey and Russia. In the United States, it is mainly distributed in western states. It is used for hay and pasture in the Northern Great Plains and the intermountain region (USDA-NRCS 2012). Tall wheatgrass has a high yield and quality but is less palatable than other wheatgrasses. It also provides food for wildlife and can be used for erosion control (USDA-NRCS 2012). Moreover, it has been reported that tall wheatgrass has the potential to serve as a source of biofuel (USDA-NRCS 2012). Tall wheatgrass is also used for plant breeding, where it provides salinity, drought, and disease resistance for wheat cultivars. It is highly adapted to saline and alkali conditions and well adapted to wet (USDA-NRCS 2012). It is less adapted to drought than crested wheatgrass. Tall wheatgrass establishes slowly, thus, grazing should be managed carefully. No pests or pathogens have been reported to attack tall wheatgrass.

Western Wheatgrass

Western wheatgrass *Pascopyrum smithii* Rydb. is a native cool season long-lived perennial grass. It is distributed through the western and midwestern regions of the United States (USDA-NRCS 2012). Western wheatgrass is widely used for erosion control in the central and Northern Great Plains. It is also a high-quality forage (USDA-NRCS 2012). The growth of western wheatgrass needs moderate to high soil moisture. It grows with many other grass species

(e.g., blue grama *Bouteloua gracilis*, buffalograss *Bouteloua dactyloides*, needlegrasses *Nassella pulchra*). The establishment of western wheatgrass can take up to several years (USDA-NRCS 2012). Grasshoppers *Melanoplus differentialis* (Thomas), ergot *Claviceps purpurea*, and stem and leaf rusts *Puccinia graminis* Pers. are the major pests and pathogens of western wheatgrass (USDA-NRCS 2012).

Meadow Brome

Meadow brome *Bromus biebersteinii* Roem & Schult. is an introduced cool season long-lived perennial grass (USDA-NRCS 2012). It originated from southeastern Asia. Meadow brome is mainly used for grazing and is palatable to all classes of livestock (USDA-NRCS 2012). Meadow brome is also a good source for erosion control due to its massive roots. It provides food and shelter for wildlife. It is cold tolerant and adapted to many kinds of soil textures, but cannot grow well in saline soil and regions with high precipitation (USDA-NRCS 2012). It recovers quickly after grazing. Silvertop *Joycea pallida* and head smut *Sphacelotheca reiliana* are common diseases of meadow brome (USDA-NRCS 2012).

Smooth Brome

Smooth brome *Bromus inermis* Leyss. is an introduced cool season long-lived perennial grass (USDA-NRCS 2012). It was introduced from Europe and Asia to the United States in the 1880s (USDA-NRCS 2012). It is distributed throughout almost all regions of the United States (USDA-NRCS 2012). Smooth brome is used for pasture grass with high protein and low crude fiber content. It is also used for erosion control due to its massive root system. Smooth brome is adapted to drought and extreme temperature, but is susceptible to some diseases in high-humidity areas (USDA-NRCS 2012). It is important to note that smooth brome is an invasive grass. Grasshopper and bromegrass seed midge *Contarinia bromicola* Marikovskij & Agafonova are its

major pests, and seedling blight *Cochliobolus miyabeanus* is a major disease (USDA-NRCS 2012).

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CHAPTER 2. CROP AND PRAIRIE GRASSES SERVING AS HOSTS FOR THE HESSIAN FLY

Introduction

Antagonistic interactions between insects and plants can be explored from two perspectives (Schoonhoven et al. 2005). The insect's perspective can be summarized by the following questions: Is the plant easy to find and identify? What resources does it offer for survival, growth, and reproduction? Does the plant defend itself against attack? Are competitors likely to be present? Does the plant provide enemy-free space? The plant's perspective can be summarized by different questions: Is attack by the insect rare or common? What is the precise nature of the attack? When does it occur during plant development? What impact does attack have on survival, growth and reproduction? Is active defense possible and is it costly? Is there time to compensate for early damage to the plant before reproduction occurs? These questions also occur within a larger context. For the insect this context is: how suitable is this plant species relative to other species that serve as hosts? For the plant this context is: how important is defense against this particular insect relative to everything else that needs to be done to achieve maximal fitness, including defense against abiotic stress and additional agents of biotic stress?

For the past seventy years, antagonistic interactions between the Hessian fly and its host plants have received significant attention from researchers (Harris et al. 2003, Stuart et al. 2012). The reason is the Hessian fly's status as a pest of wheat, *Triticum aestivum* L., one of the world's most important food crops. In turn, this pest status has meant that most research on Hessian fly-plant interactions emphasizes the plant's perspective. The consensus on Hessian fly attack being of importance to wheat production is that the Hessian fly, and gall midges in general, persist at low population levels and then, over a period of 2-3 years, build into economically significant

populations, that continue as outbreak populations for 3-7 years (Barnes 1956, Gagné 1994, Berzonsky et al. 2003). Particular regions of the world, (e.g. Morocco and the central region of the United States), have more frequent outbreaks or even permanent pest outbreaks (Ratcliffe et al. 1994, Naber et al. 2000, Ratcliffe et al. 2000, Chen et al. 2009b). Regardless of the frequency of outbreaks, impact on the plant is significant when Hessian fly attack occurs (Berzonsky et al. 2003, Anderson and Harris 2006, 2008, Anderson et al. 2011). During the seedling stage, attack halts plant growth and many plants die. During stem elongation, the presence of larvae within the stem reduces production and quality of seeds. The Hessian fly larva's impact on the seedling wheat plant is independent of pest density (Anderson and Harris 2006) and results from its feeding habit as a gall-maker. The larva induces a gall nutritive tissue at the base of the seedling plant near the meristem (Harris et al. 2006b). The nutritive tissue acts as a nutrient sink (Shorthouse and Rohfritsch 1992), robbing the plant of resources that would have been directed toward future growth.

Gall-making is a strategy used by many insect species including wasps, cecidomyiids, thrips, and aphids, as well as evolutionarily divergent organisms, notably bacteria, fungi, viruses, nematodes and mites (Gagné 1994, Stone et al. 2003, Dorchin and Freidberg 2008). For the most part it is not known how gall-makers manipulate the plant to create a tissue that benefits the gall-maker while harming their own growth and reproduction (Rohfritsch 2008, Dorchin et al. 2009), an exception being the crown gall bacterium, *Agrobacterium tumefaciens*, which secretes a series of effector proteins that force the plant to create foods that only the bacterium can feed on (Zupan et al. 2000).

Gall-making is viewed as a highly specialized interaction that limits the number of plant species that can serve as hosts to a gall-making insect (Shorthouse and Rohfritsch 1992, Stone et

al. 2003, Schoonhoven et al. 2005). For cecidomyiid gall-makers, of which the Hessian fly is one, the vast majority of species are considered to be specialist herbivores (Gagné 1994) having a small number of closely related plant species, usually within a single genus, that serve as hosts (Gagné 1994). Exceptions to the extreme specialization of cecidomyiids are the generalist species that inoculate plants with a plant pathogenic fungus and then use the fungus as food (Gagné 1994). The Hessian fly is unusual in feeding directly on the plant (rather than on a fungus) while also having a host range that extends beyond a single plant genus (Table 2). Wheat, which is the Hessian fly's best-known host, belongs to the genus *Triticum*. Most species in this grass genus have been recorded as hosts for the Hessian fly, but there also are many recorded hosts outside of the genus *Triticum*. The majority of these belong to fifteen other genera in the tribe Triticeae (Table 2). An exception to this rule is brome grass in the tribe Bromeae. Several grasses in this tribe belonging to the genus *Bromus* are listed as non-hosts in Table 3, which is taken from Zeiss et al. (1993). However, brome also appears in Table 2 as a host because at least one species can serve as a host: Hessian fly populations in New Zealand are economic pests of *Bromus willdenowii* (Prestidge 1992), and their ability to use this brome grass as a host has been confirmed in a series of greenhouse tests (Harris et al. 1996, Harris et al. 2001). In addition to there being Hessian fly hosts outside of the tribe Triticeae, it is interesting that the genus *Triticum* mostly has species that are hosts, with the exception of einkorn wheat, *Triticum monococcum*, which is not a host (Table 3).

Table 2. Grasses found to be host plants of the Hessian fly. Table was compiled by Zeiss et al. (1993), but I added to this table a number of more recent studies. The species included in my experiments are in bold.

Supertribe/tribe	Species	Common name	References
Tribe Bromeae	*Bromus willdenowii*	Brome (New Zealand)	Prestidge et al., 1992
GENUS *Bromus*			Harris et al. 1996
Tribe Poeae	*Lolium loliaceum*	Darnel ryegrass	Jones 1939
GENUS *Lolium*			
	L. remotum		Jones 1939
	L. temulentum	Darnel ryegrass	Jones 1939

Table 2 (Cont.). Grasses found to be host plants of the Hessian fly.

Supertribe/tribe	Species	Common name	References
Tribe Triticeae GENUS *Aegilops*	*Aegilops bicornis*	goatgrass	Jones 1938, Gill et al., 1985
	A. biuncialis		Jones 1938, Gill et al., 1985
	A. caudata		Gill et al. 1985
	A. columnaris		Jones 1938, Gill et al., 1985
	A. crassa	Persian goatgrass	Gill et al., 1985
	A. cylindrica	jointed goatgrass	Jones 1938
	A. kotschyi		Gill et al., 1985
	A. longissima		Gill et al., 1985
	A. ovata		Jones 1938, Stokes 1957, Gill1985
	A. sharonensis		Gill et al., 1985
	A. speltoides	goatgrass	Gill et al., 1985
	A. tauschii	goatgrass	Jones 1938, Gill et al., 1986
	A. triuncialis	barb goatgrass	Jones 1938, Gill et al., 1985
	A. triaristata		Jones 1938, Gill et al., 1985
	A. umbellulata		Gill et al., 1985
	A. variabilis		Gill et al., 1985
	A. ventricosa		Jones 1938
GENUS × *Agrohordeum*	× *Agrohordeum macounii*	Macoun wild rye (*Elyhordeum macounii*)	Jones 1939
GENUS *Agropyron*	***Agropyron cristatum***	**crested wheatgrass**	Jones 1939
	A. dasystachyum	thickspike wheatgrass	Jones 1939
	A. desertorum	desert wheatgrass	Jones 1939
	A. fragile	Siberian wheatgrass	Jones 1939
	A. repens	quackgrass	Hayhurst 1909, Noble 1931, Rockwood & Reeeher 1933, Jones 1939, Harris et al. 1996 (NZ)
	A. semicostatum	drooping wild rye	Jones 1939
	A. spicatum	bluebunch wheathgrass	Jones 1939
	A. subsecundum	slender wheatgrass	Jones 1939
	A. trachycaulum	Slender wheatgrass	Jones 1939
GENUS *Amblyopyrum*	*Amblyopyrum muticum*	amblyopyrum	Gill et al. 1985
GENUS *Dasypyrum*	*Dasypyrum villosum*	mosquitograss	Jones 1939
GENUS *Elymus*	***Elymus canadensis***	**Canada wild rye**	Noble 1931, Jones 1939
	E. caninus	beared wheatgrass	Jones 1939
	E. ciliaris		Jones 1939
	E. condensatus	giant wild rye	Jones 1939
	E. dahuricus		Jones 1939
	E. elymoides	squirreltail	Jones 1939
	E. sibiricus	Siberian wildrye	Jones 1939
	E. triticoides	alkali rye	Jones 1939
	E. villosus	silky wild rye	Jones 1939
	E. virginicus	Virginia wild rye	Jones 1939
	E. pungens		Jones 1939
	E. repens	quackgrass	Harris et al. 1996 (NZ) Hayhurst 1909, Noble 1931, Rockwood & Reeeher 1933, Jones 1939, Harris et al. 1996 (NZ)
GENUS *Elytrigia*	*E. strigosa*		Jones 1939
GENUS *Hordeum*	*Hordeum bulbosum*	bulbous barley	Jones 1939
	H. jubatum	foxtail barley	Jones 1939
	H. murinum	mouse barley	Jones 1939 Harris et al. 1996 (NZ)
	H. pusillum	little barley	Jones 1939
	H. secalinum	meadow barley	Jones 1939
	H. spontaneum	wild barley	Jones 1936, Hill et al. 1952

Table 2 (Cont.). Grasses found to be host plants of the Hessian fly.

Supertribe/tribe	Species	Common name	References
GENUS *Hordeum*	*H. vulgare*	common barley	McColloch and Salmon 1918, McColloch 1923, Hill et al. 1952 Stokes 1957, Morrill 1982
GENUS *Hystrix*	*Hystrix californica*		Jones 1939
GENUS *Leymus*	*Leymus racemosus*	mammoth wildrye	Jones 1939
	L. secalinus	wild rye	Jones 1939
GENUS *Psathyrostachys*	*Psathyrostachys juncea*	Russian wild rye	Jones 1939
GENUS *Pascopyrum*	***Pascopyrum smithii***	**Western wheatgrass**	
GENUS *Secale*	*Secale cereale*	cereal rye	Cartwright 1992, Stokes 1957, Morrill 1982
GENUS *Thinopyrum*	***Thinopyrum intermedium***	**Intermediate wheatgrass**	Johnson et al. 1987 Jones 1939
	Thinopyrum ponticum	**Tall wheatgrass**	Jones 1939
	Triticum aestivum	common wheat	Many; the principal host
	T. compactum	clubbed wheat	Stokes 1957
	T. dicoccoides	wild emmer	Stokes 1957
	T. dicoccum	dicoccum wheat	McColloch and Salmon 1918, McColloch 1923
	T. durum	durum wheat	McColloch and Salmon 1918, McColloch 1923, Stokes 1957
	T. polonicum		McColloch and Salmon 1918
	T. spelta	spelt	McColloch and Salmon 1918, McColloch 1923, Stokes 1957
GENUS Triticum	*T. turgidum*	rivet wheat	McColloch and Salmon 1918, Stokes 1957

Table 3. Grasses on which experiments have failed to detect Hessian fly reproduction. Table was compiled by Zeiss et al. (1993).

Subfamily	Supertribe/tribe	Species	Common name	References
SUBFAMILY CHLORIDOIDEAE	Tribe Chlorideae	*Chloris verticillata*	tumble windmill grass	Jones 1939
	Tribe Eragrosteae	*Eragrostis cilianensis*	stinkgrass	Jones 1939
		Eragrostis trichodes	sand lovegrass	Jones 1939
		Muhlenbergia racemosa	marsh muhly	Jones 1939
		Muhlenbergia schreberi	nimblewill	Jones 1939
		Sporobolus asper	tall dropssed	Jones 1939
		Tridens flavus	purpletop tridens	Jones 1939
		Digitaria ischaemum	smooth crabgrass	Jones 1939
		Digitaria sanguinalis	hairy crabgrass	Jones 1939
		Echinochloa crus-galli	Barnyardgrass	Jones 1939
		Panicum dichotomiflorum	fall panicgrass	Jones 1939
		Panicum virgatum	switchgrass	Jones 1939
		Paspalum setaceum	thin paspalum	Jones 1939
		Setaria glauca	yellow foxtail	Jones 1939
		Andropogon furcatus	big bluestem	Jones 1939
		Andropogon scoparius	little bluestem	Jones 1939
		Sorghastrum nutans	indiangrass	Jones 1939
		Tripsacum dactyloides	eastern gamagrass	Jones 1939
SUBFAMILY POOIDEAE	Supertribe Poanae, Tribe Aveneae	*Agrostis gigantea*	redtop	Forbes 1891, Gossard and Houser 1906, Jones 1939, Stokes 1957
		Alopecurus sp.		Forbes 1891, Gossard and Houser 1906
		Alopecurus pratensis	meadow foxtail	Marchal 1897

Table 3 (Cont.). Grasses on which experiments have failed to detect Hessian fly reproduction.

Subfamily	Supertribe/tribe	Species	Common name	References
		Avena fatua	wild oat	Stokes 1957
		Avena sativa	common oat	McColloch and Salmon 1918, Cartwright 1922, McColloch 1923, Jones 1939, Stokes 1957, Morrill 1982, Harris and Rose 1991
		Avena sterilis	sterile oat	Stokes 1957
		Holcus lanatus	common velvetgrass	Marchal 1987
		Koeleria macrantha	prairie Junegrass	Jones 1939
		Phleum pratense	timothy	Gossard and Houser 1906, Jones 1939, Stokes 1957
		Sphenopholis obtusata	prairie wedgescale	Jones 1939
	Supertribe Poanae, Tribe Poeae	*Dactylis glomerata*	orchardgrass	Forbes 1891, Marchal 1897, Gossard and Houser 1906
		Festuca pratensis	meadow fescue	Marchal 1897, Stokes 1957,
		Lolium sp.		Jones 1939
		Lolium perenne	perennial ryegrass	Jones 1939
		Poa sp.		Gossard and Houser 1906
		Poa compressa	Canada bluegrass	Jones 1939
		Poa pratensis	Kentucky bluegrass	Jones 1939
	Supertribe Poanae, Tribe Stipeae	*Stipa spartea*	porcupinegrass	Jones 1939
	Supertribe Triticanae, Tribe Bromeae	*Bromus catharticus*	rescuegrass	Jones 1939
		B. commutatus	bald brome	Marchal 1897
		B. japonicus	field brome	Jones 1939
		B. mollis	soft brome	Jones 1939
		B. secalinus	rye brome	Jones 1939
		B. sterilis	poverty brome	Jones 1939
		B. tectorum	Cheatgrass	Jones 1939
		B. biebersteinii	**Meadow brome**	
		B. inermis	**Smooth brome**	
	Supertribe Triticanae, Tribe Triticeae	*Triticum monococcum*	einkorn	McCollloch and Salmon 1918, McColloch 1923

Systematists specializing in the family Cecidomyiidae are skeptical about the idea that the Hessian fly has a relatively broad host range (R. Gagné, Smithsonian Museum, personal communication to M. Harris). In some cases, they dismiss the evidence, especially when it consisted of field observations where something that looked like a Hessian fly larva was found

attacking a wild grass (Barnes 1956). Cecidomyiid larvae are very difficult to identify using morphological traits and need to be reared through to the adult stage to accurately identify to the species level (Gagné 1994). Other evidence that is harder to dismiss comes from experiments testing whether Hessian fly females from laboratory colonies lay eggs on other grasses and whether their offspring survived to produce adults (Jones 1936, 1938, 1939).

The question at the center of my research was: how do crop and non-crop grasses compare as hosts for the Hessian fly and in their response to Hessian fly attack? I chose seven native and introduced prairie grasses (Table 4) that are significant features of the grasslands of the Northern Great Plains and therefore form the landscape or matrix in which many crop grasses, including wheat, are produced. Previous studies on Hessian fly interactions with these prairie grasses (Jones 1936, 1938, 1939) provided yes/no answers to a number of questions including: Is the species accepted as a host by ovipositing females? Are offspring able to develop through to the adult stage? I looked at these questions in greater detail and also posed questions relevant to the plant's perspective: What impact does attack have on the plant's survival, growth and reproduction? Is the plant able to compensate for the damage that results from attack? Three crop grasses served as controls: wheat the preferred host, barley a host less preferred for egglaying and less suitable for offspring, and oat a non-host even less preferred for egglaying and entirely unsuitable for offspring (Harris et al. 2001).

Materials and Methods

Insects

The Hessian fly strain 'Great Plains' was used in experiments. The colony of this strain is maintained at North Dakota State University and originated from ca. 5000 puparia obtained in

Table 4. Ten crop and non-crop grass species used in tests to explore insect-plant relationships with the Hessian fly.

Tribe	Scientific name	Common name	Accession #	Cultivar	Center of Origin	Usage
Triticeae	*Triticum aestivum* L.	Wheat	NA	'Newton'	Introduced (Eurasia)	Cereal crop
Triticeae	*Hordeum vulgare* L.	Barley	NA	'Robust'	Introduced (Eurasia)	Cereal crop
Triticeae	*Elymus canadensis* L.	Canada wild rye	NA	'Mandan'	Native	Restoration; Grazing; Wildlife
Triticeae	*Agropyron cristatum* L.	Crested wheatgrass	NA	'Nordan'	Introduced (Eurasia)	Grazing/hayland; Erosion control
Triticeae	*Thinopyrum intermedium* Barkworth & D.R. Dewey	Intermediate wheatgrass	NA	'Reliant'	Introduced (Eurasia)	Erosion control; Wildlife; Plant breeding (resistance to BYDV[1])
Triticeae	*Thinopyrum ponticum* Podp.	Tall wheatgrass	98526	NA	Introduced (Eurasia)	Grazing/hayland; Erosion control; Biofuel; Nutrient removal; Plant breeding
Triticeae	*Pascopyrum smithii* Rydb.	Western wheatgrass	477993	'Rodan'	Native	Erosion control; Grazing; Reclamation
Bromeae	*Bromus biebersteinii* Roem. & Schult.	Meadow brome	9058933	'Fleet'	Native	Grazing; Erosion control; Wildlife
Bromeae	*Bromus inermis* Leyss.	Smooth brome	9023426	'Rebound'	Native	Grazing; Erosion control; Wildlife
Poeae	*Avena sativa* L.	Oat		'Morton'	Introduced (Eurasia)	Cereal crop

[1]Barley Yellow Dwarf Virus, a plant virus that infects barley, wheat, maize and rice.

2000 from the USDA-ARS Laboratory at Purdue University. The 'Great Plains' strain of the Hessian fly is *avirulent* for the 33 known *H* resistance genes, i.e. it does not survive on wheat genotypes that contain any of the 33 known resistance genes (Stuart et al. 2012). The Hessian fly colony was reared on the susceptible hard red spring wheat cultivar 'Reeder'. Wheat was grown in plastic pots (diameter 15.2 cm, depth 16 cm) in the greenhouse (temperature of 20 ± 2 ºC, 30-60% RH and photoperiod of 16:8 light:dark). Plants were infested at the two-leaf stage by being exposed to 100 mated females for 24 hours, starting at 1000 hours when most newly emerged adult females have mated but not yet started to oviposit (Harris and Rose 1991, Harris et al. 2001). Forty-eight hours later, egg-infested plants were moved to an environmental chamber with high humidity (20 ºC, 70-80% RH and photoperiod of 16:8 light:dark cycle). High humidity aids both egg hatch and larva migration to the plant base where feeding occurs. After 48 hours at this higher humidity, plants were moved back to the greenhouse, where they remained for approximately two weeks. At this time the feeding stage was finished and pupae were forming. Plants were moved to an environmental chamber (24º C, 70% RH, and 12:12 light:dark cycle). A little over a week later (7-10 days), Hessian fly adults began to eclose. Most males emerge in the late hours of the afternoon, with a smaller number eclosing soon after dawn, which also is when adult females eclose (Bergh et al.1990).

Plants

Ten grass species were used in the experiments (Table 4). Prairie grass seeds were obtained from the USDA-NRCS Plant Material Center in Bismarck, North Dakota. Wheat, barley and oat seeds were obtained from plant breeders at North Dakota State University.

Seeds were planted in cone-tainers (4 cm diameter by 21 cm depth; Stuewe & Sons, Corvallis, OR) filled with potting soil (Sunshine SB100 Mix, Sungro Horticulture Distribution

Inc., Bellevue, WA). Cone-tainers were held in racks (7×14 cells, Stuewe & Sons, Corvallis, OR), which were held in the greenhouse (temperature of 20 ± 2º C, 30-60% RH and photoperiod of 16:8 light:dark cycle). Since the ten grass species grew at different rates but needed to be in the same developmental stage, (i.e. the two-leaf stage, at the time of experiments), preliminary studies were conducted to determine how many days of growth were needed to create a two-leaf plant (Table 5). For each block of the experiment, the ten grass species were planted (28 plants per day) at the requisite times (Table 5). Plants were watered daily and fertilized once a week (Jack's Professional 20:20:20 N-P-K Fertilizer, J.R. Peters Inc., Allentown, PA).

Oviposition Preference Tests

Test One was conducted in late 2010, with 11 blocks run on 11 different days: December 7, 8, 10, 11, 13, 17, 18, 19, 20, 22, and 23. Each block used a different group of females. For each block, one plant of each species was placed randomly in a circular array (40 cm diameter, 13.9 ± 1 cm distance between cones) in soil held in a pot (60 cm diameter, 60 cm depth, Stuewe & Sons, Corvallis, OR). The array of ten plants was covered with a cylindrical cage (60 cm diameter, 36 cm high), with a mesh ceiling and walls of blue construction paper. Ten mated females were released into the cage at 1000 hours. By 1600 hours, adult females were dead. Plants were removed from the cage and eggs were counted. Eggs were recorded separately for the two leaves, with placement on the abaxial or adaxial leaf surface also noted.

Test Two was similar in all respects to Test One except that the ovipositing females were presented with just the seven pasture grasses. Absent from the cage were the crop grasses, wheat, barley, and oat. There were two reasons for this. First, the crop species are larger than the pasture grasses in terms of leaf area (Table 5), which shows a positive association with visual attraction of ovipositing Hessian fly females (Harris et al. 1993). Second, because the crop

Table 5. Days of growth required for each of the ten grasses to attain the two-leaf seedling stage and the size of two-leaf seedlings at the time of testing. Within each column, means that do not share a letter are significantly different (ANOVA and means separation by Tukey-Kramer HSD test at P <0.05).

Species	Days to two leaf stage	1st leaf		2nd leaf		Total 1st and 2nd leaf	
		Leaf length (cm) $\bar{X}$ ± SE	Leaf area (cm²) $\bar{X}$ ± SE	Leaf length (cm) $\bar{X}$ ± SE	Leaf area (cm²) $\bar{X}$ ± SE	Leaf length (cm) $\bar{X}$ ± SE	Leaf area (cm²) $\bar{X}$ ± SE
Wheat	7	11.73 ± 0.91 a	4.79 ± 0.37 c	16.27 ± 0.61 a	6.04 ± 0.38 a	28.00 ± 1.06 a	10.83 ± 0.58 b
Barley	10	11.40 ± 0.40 a	7.71 ± 0.45 a	13.90 ± 0.88 abc	7.60 ± 0.74 a	25.30 ± 1.03 ab	15.31 ± 1.05 a
CR (Canada wildrye)	18	7.04 ± 0.47 c	0.78 ± 0.07 d	12.09 ± 0.67 abc	1.95 ± 0.22 b	19.13 ± 0.94 c	2.73 ± 0.24 b
CW (crested wheatgrass)	18	6.40 ± 0.49 c	0.53 ± 0.05 d	11.63 ± 0.60 bc	1.08 ± 0.08 b	18.03 ± 0.90 c	1.62 ± 0.11 c
IW (intermediate wheatgrass)	15	10.25 ± 0.59 ab	1.43 ± 0.13 d	13.81 ± 0.79 abc	2.19 ± 0.19 b	24.07 ± 0.90 b	3.62 ± 0.25 c
TW (tall wheatgrass)	18	11.52 ± 0.50 a	1.38 ± 0.14 d	14.85 ± 1.09 ab	1.85 ± 0.19 b	26.37 ± 1.17 ab	3.23 ± 0.29 c
WW (Western wheatgrass)	23	8.20 ± 0.50 bc	0.76 ± 0.06 d	10.52 ± 1.55 c	1.06 ± 0.19 b	18.72 ± 1.48 c	1.83 ± 0.20 c
MB (meadow brome)	15	7.24 ± 0.39 c	1.11 ± 0.10 d	13.49 ± 0.73 abc	2.06 ± 0.26 b	20.73 ± 1.00 c	3.17 ± 0.35 c
SB (smooth brome)	15	6.61 ± 0.42 c	1.06 ± 0.09 d	11.51 ± 0.64 bc	2.27 ± 0.21 b	18.12 ± 0.83 c	3.33 ± 0.27 c
Oat	10	11.61 ± 0.44 a	6.22 ± 0.33 b	13.15 ± 1.26 abc	6.23 ± 0.75 a	24.76 ± 1.15 b	12.45 ± 0.59 b
		F = 18.77	F = 69.07[1]	F = 4.75[1]	F = 31.01[1]	F = 12.65	F = 73.04[1]
		d.f. = 9, 109	d.f. = 9, 109	d.f. = 9, 109	d.f. = 9, 109	d.f. = 9, 109	d.f. = 9, 109
		P < 0.0001	P < 0.0001	P =0.0002	P < 0.0001	P < 0.0001	P < 0.0001

[1]Welch ANOVA was used for the comparing means.

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grasses received the majority of eggs in Test One, it was hoped that removing these crop grasses would accentuate differences in oviposition responses to the non-crop grasses. Test Two was conducted in early 2011, with 10 blocks run on May 11, 13, 14, 15, 16, 18, 21, 22, 23, and 25.

For oviposition preference tests, a one-way ANOVA was run using log- transformed data from Test One and using square root transformed data from Test Two. Data for assessing relative attractiveness across the grass species were total eggs per plant per block, there being 11 blocks for Test One and 10 blocks for Test Two. Given a significant result from the ANOVA, the Tukey-Kramer HSD test was used to separate mean eggs at $P < 0.05$. Differences in the distribution of eggs within plants also were tested by a one-way ANOVA, either using percentages of eggs on the abaxial versus the adaxial leaf surface or eggs on the first versus second leaf. For the latter comparison, data were square root transformed.

Offspring Performance Tests

The test began in late 2010, with different blocks of plants infested with eggs on different days: December 7, 8, 9, 10, 17, 19, 22, and 26. Each block used different groups of females. Seven of the ten grass species (wheat, barley, CR, CW, IW, TW, and WW) were included in the test. Oat was excluded because it is known to not be a host (Harris *et al*., 2001). Smooth brome and meadow brome were excluded because, in preliminary tests, all larvae died within five days of initial attack. For each block, seeds of the seven species were planted individually in cone-tainers (n = 14), and randomly assigned a number from 1 to 14 (Figure 2). When seedlings had reached the two-leaf stage, 12 of the 14 plants were exposed to ovipositing females (n = 50-100) for six hours. To minimize differences in eggs across the seven different species, some of which are more attractive to ovipositing females than others, individual plants were removed at intervals and eggs were counted under microscope (20X). If the egg count was below 15, the

plant was placed back in the cage for further infestation. If the egg count was above 15 eggs, the plant was considered to have enough eggs and was not returned to the cage. From the results of preliminary tests, we predicted that the crop species would be the first to receive eggs and therefore checked these species first for eggs. The infestation of each block of plants lasted approximately six hours. Plants that did not acquire enough eggs during that time were discarded. After receiving eggs, the eggs on each plant were counted under microscope (20X). The remaining two of the fourteen plants served as non-attacked controls and were not exposed to females (Figure 2). After plants assigned to the attacked treatment were infested with eggs, plants were held in the greenhouse for 48 hours (temperature of 20 ± 2º C, 30-60% RH and photoperiod of 16:8 light:dark cycle) and then during larval eclosion and migration were held for 24 hours in a high humidity chamber (20 ± 1º C, 70-80% RH and photoperiod of 16:8 light:dark cycle). At the end of 24 hours, all viable eggs have hatched (Harris et al. 2001). Eggs that had not hatched were counted under the microscope (20X) and plants were moved back to the greenhouse, where they remained until destructive sampling was conducted.

Nine days after the initiation of larval attack (hereafter referred to as 'post-attack'), four of the 12 attacked plants were measured for leaf growth and destructively sampled to count larvae (Figure 2). First, the leaf blade of the third and fourth leaves was measured from the ligule to the tip of the leaf. If the ligule of the fourth leaf was visible, the leaf blade was measured from the ligule to the tip of the leaf. However, if the ligule of the fourth leaf was enclosed in the sheaths of older leaves, the blade was measured from the point of its emergence from the sheath to its distal tip (Harris et al. 2006a). After measuring the leaves, each leaf was removed to reveal larvae at the base of the plant. Numbers of dead and living larvae were recorded (Figure 2).

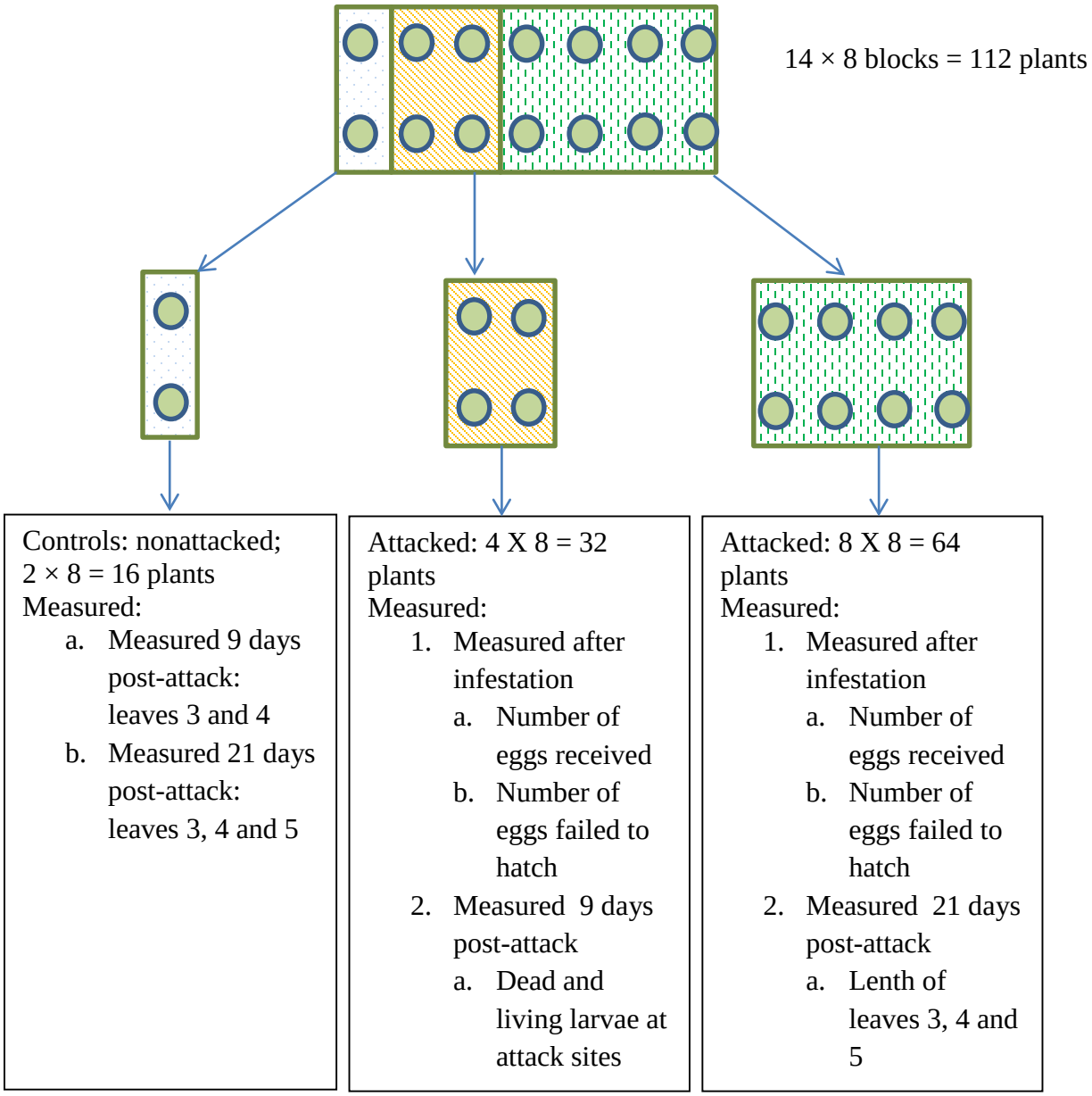

Figure 2. Experimental design for measuring Hessian fly performance on the seven grasses and response of each grass to attack by Hessian fly larvae. Each represents a single plant.

At this time, living larvae were white and had grown to the second instar (Gagné and Hatchett 1989). Dead larvae were still red, like newly eclosed larvae, and had not grown. Some of the plants were damaged by fungus gnats, which eat both plant tissue and Hessian fly pupae, and was discarded.

The other eight of the 12 attacked plants (Figure 2), as well as the two control plants, were held in the greenhouse for an additional 13 days, i.e. until 21 days post-attack. At this time, larvae have finished feeding and entered the third instar, which does not feed and is encased in the puparium. Each plant was removed from the soil, and the lengths of the blade of all the leaves except the first and the second (whose growth is not impacted by larval attack, Anderson and Harris 2006) were measured. After these leaf measurements, the plant was trimmed of its leaves and roots, leaving only 5 cm of the leaf sheaths (where the pupae are located) and 0.5 cm of the roots, and placed in a glass vial (3 cm diameter × 8 cm long) containing a 2 cm layer of moist sand. A lid (1.5 cm diameter) with a mesh insert closed the vial, which was then placed in a controlled climate chamber (24º C, 70% RH, and 12:12 L:D). Vials were checked each day for newly eclosed adults (between 0900 hours and 2100 hours). Newly eclosed adults were removed from the vial and moved to a vial containing 70% ethanol. Recorded on the label was: date of emergence, plant treatment, numbers of male and female. After checking vials each day, water was added if the sand was dry.

The reproductive potential of males and females was estimated by measuring wing length using the method of (Bergh et al. 1990). The right wing was positioned on the moistened surface of a glass slide (Figure 3), ventral side up and perpendicular to the body, with the radial sector vein aligned along an ocular stage micrometer (Mitutoyo, Kawasaki, Japan). The distance was measured to the nearest 0.1 mm, starting from the proximal end of the axillary sclerite and ending at the point where the radial vein terminates at the wing's distal edge (Figure 3).

For the offspring performance test, significant differences ($P < 0.05$) in insect and plant responses across the seven grasses were tested by one-way ANOVA when data met the assumptions of homogeneous variances and normal distribution. A Welch ANOVA was used

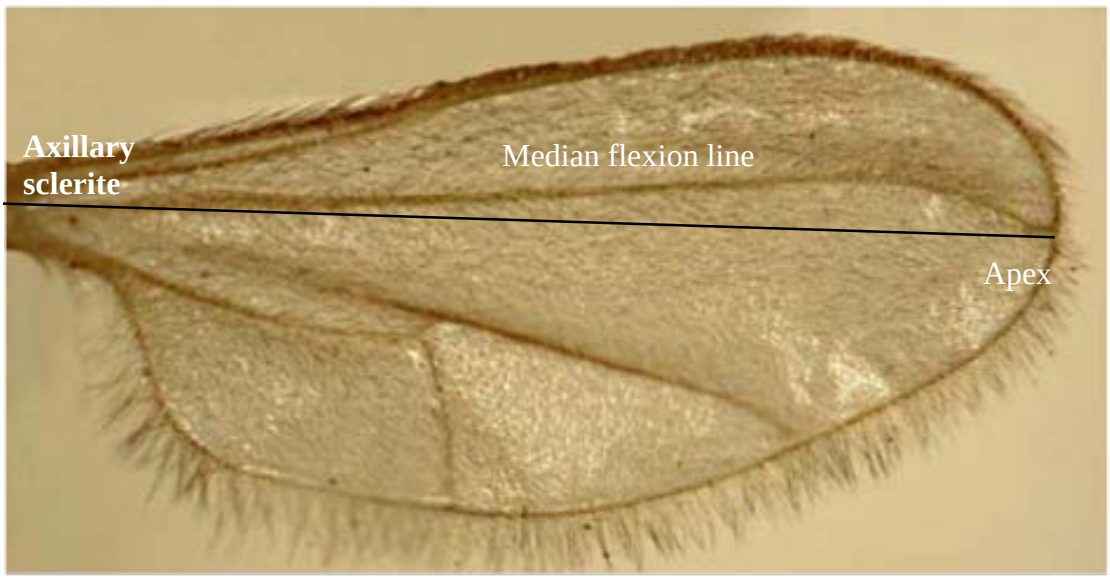

Figure 3. Features of the Hessian fly wing used to measure wing length of adult males and females. The measurement started at the proximal edge of the axillary sclerite and ended at the point where the radial vein terminated at the wing's distal edge.

when these assumptions were not met and problems with data could not be rectified by transformation (JMP 8.0.2, SAS Institute, Cary, NC, USA, 2008). When the ANOVA or Welch ANOVA showed significant differences, the Tukey-Kramer HSD test was used to establish differences between means ($P < 0.05$). Adult survival was calculated in two ways. For the first, I used data from all plants, that is the plants that produced adults and the plants that did not produce adults, the latter thus having zero survival. For the second, I only used data from plants that produced adults. Correlation analysis was used to determine relationships across the seven grasses between mean female and mean male wing length and between mean female and mean male developmental time. For responses of plants to attack, all plants sampled at nine days post-attack had a third leaf. Therefore these data were tested by one-way ANOVA. However, many attacked plants did not produce a fourth leaf. Therefore I first calculated the percentage of plants that produced a fourth leaf in each block and compared the percentage/block across the seven grasses using one-way ANOVA. The next comparison was only for the plants that had produced

a fourth leaf, data being the length of that fourth leaf and analysis by one-way ANOVA. For plants that were sampled 21 days post-attack, again only some of the attacked plants had produced a fourth leaf, and this was also the case for the fifth leaf. Thus once again I compared the proportion of plants that produced the fourth or fifth leaf using a one-way ANOVA and compared the length of these two leaves using a one-way ANOVA.

General Statistical Methods

Data were analyzed using JMP version 8.0.2 (2008; SAS Institute, Cary, NC, USA). Prior to analysis by ANOVA, data were tested ($P < 0.05$) for homogeneity of variance via O'Brien's test and normal distribution via the Shapiro-Wilk *W* test. If variances were heterogeneous or data were not normally distributed, data were transformed and retested. If data transformation did not solve the problem, I used the Welch ANOVA, which is valid for data that do not meet the assumptions for the standard ANOVA (JMP Manual, SAS Institute, Cary, NC, USA). When the data met the assumptions, ANOVAs were used to test for plant species or treatment effects. When the ANOVA showed significant differences across plant species or attacked versus non-attacked treatments, the Tukey-Kramer HSD test was used to compare means at $P < 0.05$ (JMP, SAS Institute, Cary, NC, USA).

Results

Oviposition Preference

Numbers of eggs oviposited on plants (Figure 4A) were significantly different across the ten grass species (one-way ANOVA on log transformed data: $F = 25.34$, d.f. = 9, 100, $P < 0.0001$). Wheat and barley received significantly more eggs than the other eight grasses. Among the prairie grasses, intermediate wheatgrass (IW), tall wheatgrass (TW) and western wheatgrass (WW) received more eggs than meadow brome (MB) and smooth brome (SB) (Tukey-Kramer

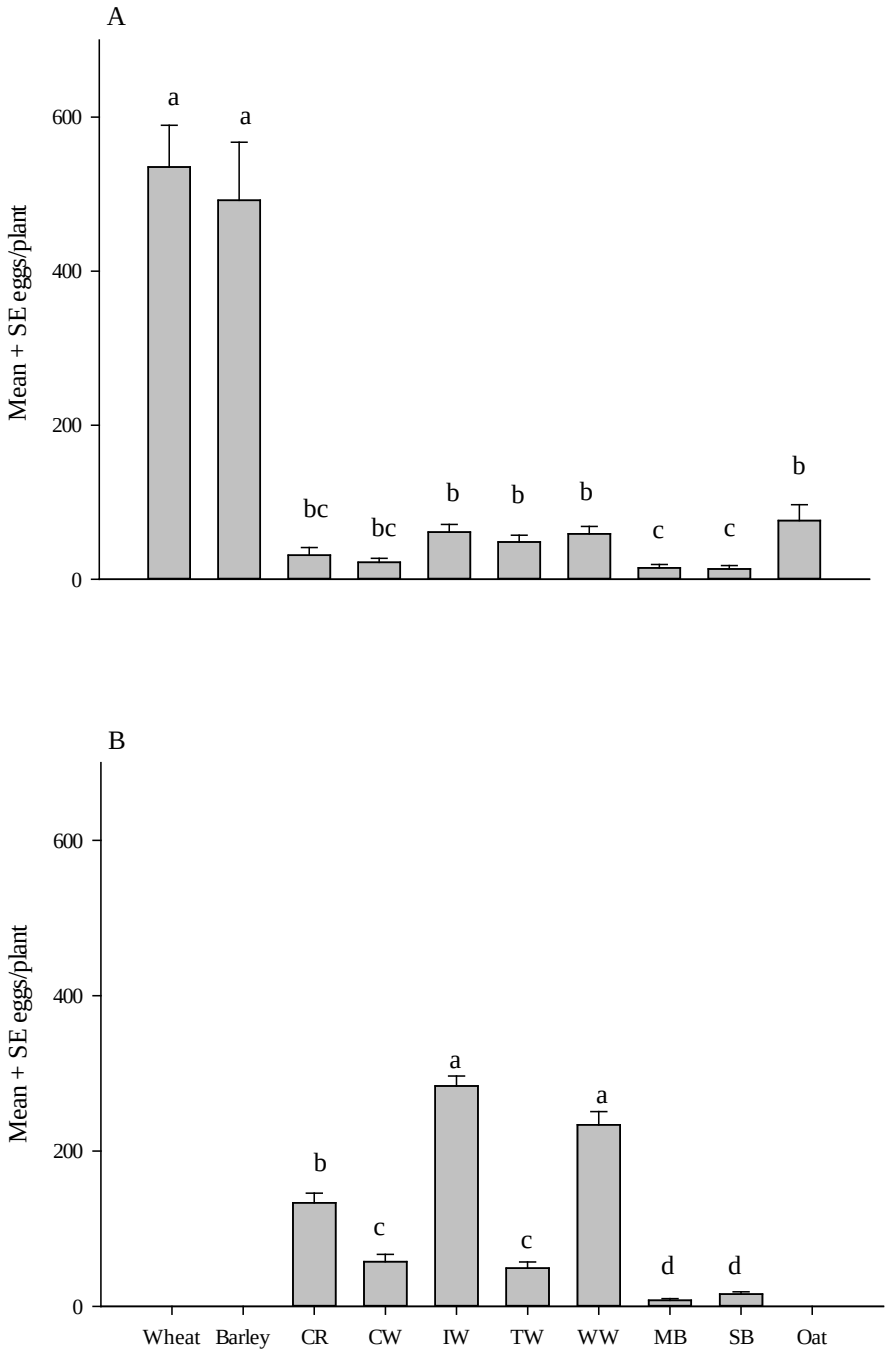

Figure 4. Eggs oviposited by Hessian fly females on two-leaf plants of the grasses presented in choice tests. (A) Choice test presenting ten crop and prairie grasses; eggs/block (mean ± SE) = 1353 ± 354 (11 blocks). (B) Choice test presenting only the seven prairie grasses; eggs/block (mean ± SE) = 781± 50 (10 blocks). Within each figure, means that do not share a letter are significantly different (ANOVA on log transformed data in (A) and on square root transformed data in (B); means separation by Tukey-Kramer HSD test at $P < 0.05$).

HSD at $P < 0.05$).

On wheat seedlings, ovipositing Hessian fly females select specific locations, with most eggs placed on the youngest leaf of the seedling and on the adaxial leaf surface (Harris and Rose 1989). I tested whether this selective placement of eggs within the seedling plant also occurs when eggs are placed on prairie grasses. The percentage of eggs placed on the younger leaf (Table 6) differed across the ten grasses (one-way ANOVA on square root transformed data: $F = 4.51$, d.f. = 9, 100, $P < 0.0001$), with a lower percentage for western wheatgrass (WW, 36%) than for wheat (69%), Canada wildrye (CR, 92%), and the two brome grasses (MB and SB, 78-79%). The percentage of eggs placed on the seedling's adaxial leaf surface (Table 6) also differed across the ten grasses (one-way ANOVA: $F = 3.10$, d.f. = 9, 100, $P = 0.0026$) with a lower percentage for barley (63%) than for tall wheatgrass (TW, 90%) and western wheatgrass (WW, 91%).

Table 6. Choice of egglaying sites by Hessian fly females ovipositing on the ten grasses. Within each column, means that do not share a letter are significantly different (ANOVA on log transformed data in younger leaf and on square root transformed data in adaxial surface; means separation by Tukey-Kramer HSD test at $P < 0.05$).

Species	Younger (2^{nd}) leaf[1] mean ± SE % eggs	Adaxial leaf surface[2] mean ± SE % eggs
Wheat	68.73 ± 2.31 ab	84.75 ± 1.44 ab
Barley	51.36 ± 4.24 abc	63.27 ± 4.23 b
CR	92.07 ± 3.42 a	88.46 ± 6.36 ab
CW	60.69 ± 9.85 abc	88.56 ± 9.01 ab
IW	60.38 ± 6.16 abc	86.05 ± 3.13 ab
TW	49.97 ± 8.90 bc	90.47 ± 2.98 a
WW	36.41 ± 7.68 c	91.20 ± 3.96 a
MB	77.87 ± 8.42 ab	65.95 ± 1.05 ab
SB	79.25 ± 5.39 ab	89.47 ± 5.99 ab
Oat	49.94 ± 9.37 bc	84.33 ± 3.10 ab

[1]Eggs on younger leaf / total eggs on plant x 100
[2]Eggs on the adaxial leaf surface / total eggs on plant x 100

A second test was conducted to determine if Hessian fly females distinguish between the less preferred prairie grasses when the more preferred crop grasses are absent. Numbers of eggs

oviposited on individual plants (Figure 4B) were significantly different across the seven prairie grass species (one-way ANOVA on square root transformed data: $F = 116.02$, d.f. = 6, 62, $P < 0.0001$). Intermediate wheatgrass (IW) and western wheatgrass (WW) received more eggs than Canada wildrye (CR), which received more eggs than crested wheatgrass (CW) and tall wheatgrass (TW). The two brome grasses (MB and SB) received few eggs (Tukey-Kramer HSD at $P < 0.05$). Percentages of eggs placed on the younger leaf differed across the seven grasses (Welch ANOVA: $F = 2.49$, d.f. = 6, 63, $P = 0.048$), as did percentages of eggs placed on the adaxial leaf surface (one-way ANOVA on square root transformed data: $F = 2.74$, d.f. = 6, 63, $P = 0.02$).

Offspring Performance

Total eggs/plant after infestation and eggs that had not hatched five days later were counted for all plants, i.e. those sampled at nine days post-attack and plants sampled at 21 days post-attack (Table 7). The number of eggs received during infestation differed across grass species (one-way ANOVA on square root transformed data: $F = 22.28$, d.f. = 6, 558, $P < 0.0001$). The number of eclosed larvae per plant, which was calculated as # larvae eclosing from eggs/plant = total # eggs/plant – # eggs not hatching/plant, also differed across grass species (one-way ANOVA: $F = 14.94$, d.f. = 6, 558, $P < 0.0001$).

Percentage survival during the egg stage differed across the seven species (Figure 5, one-way ANOVA on square root transformed data: $F = 4.02$, d.f. = 6, 558, $P < 0.0001$). The percentage of survival on intermediate wheatgrass (IW) was lower than that on western wheatgrass (WW) (Tukey-Kramer HSD at $P < 0.05$). There was no difference between survival on wheat versus survival on the other six grasses (Tukey-Kramer HSD at $P < 0.05$).

Table 7. For tests of performance, differences in Hessian fly density on grasses. Within each column, means that do not share a letter are significantly different (ANOVA on square root transformed data in total eggs and one-way ANOVA in # larvae migrating; means separation by Tukey-Kramer HSD test at P <0.05).

Species	Total eggs mean ± SE	# larvae migrating to attack sites mean ± SE
Wheat	33.74 ± 1.66 a	25.78 ± 1.34 a
Barley	36.00 ± 2.40 a	25.60 ± 1.40 a
CR	20.00 ± 1.22 cd	15.51 ± 1.08 cd
CW	15.64 ± 1.24 d	12.87 ± 1.06 d
IW	32.00 ± 2.17 ab	20.75 ± 1.25 ab
TW	24.88 ± 1.55 bc	18.62 ± 1.14 bc
WW	25.56 ± 1.81 bc	22.75 ± 1.72 ab

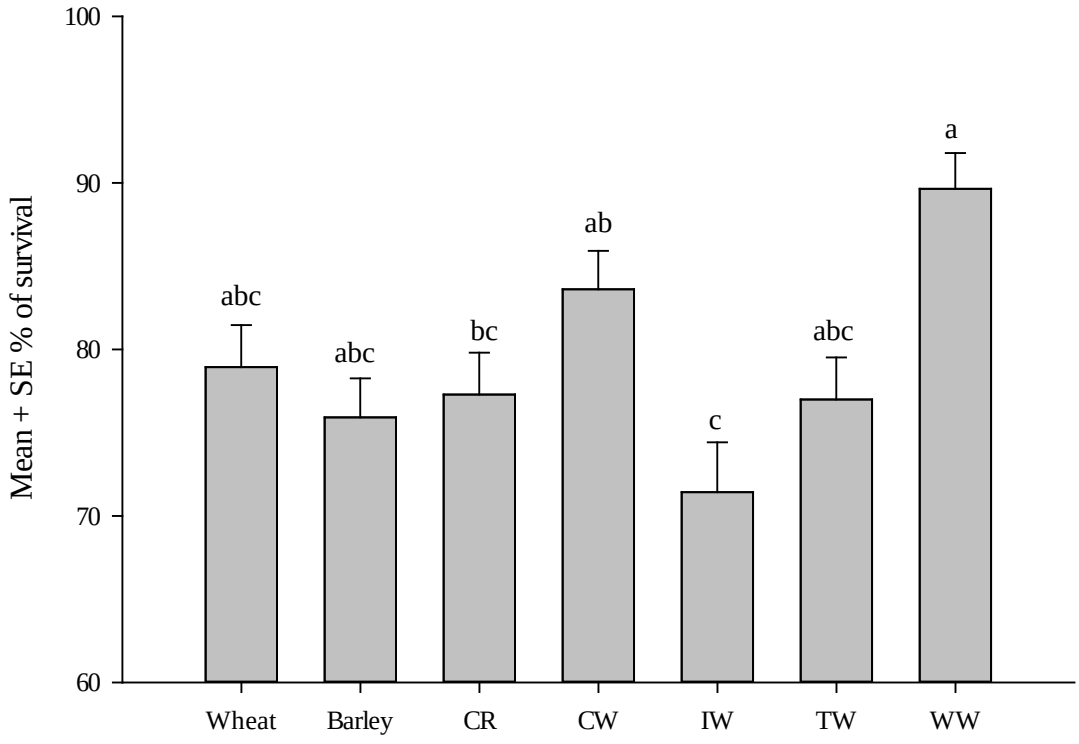

Figure 5. Hessian fly survival from egg deposition on leaf to eclosion of larvae from the egg on seven grasses. A total of 11347 larvae eclosed from a total of 15150 eggs. Means that do not share a letter are significantly different (one-way ANOVA on square root transformed data; means separation by Tukey-Kramer HSD test at P < 0.05).

Percentages of plants with living larvae found nine days after initial attack were significantly different across the seven grass species (Figure 6A, one-way ANOVA: F = 28.37,

d.f. = 6, 45, P < 0.0001), with higher percentages for wheat, barley, Canada wildrye (CR) and western wheatgrass (WW) than for crested wheatgrass (CW), intermediate wheatgrass (IW) and

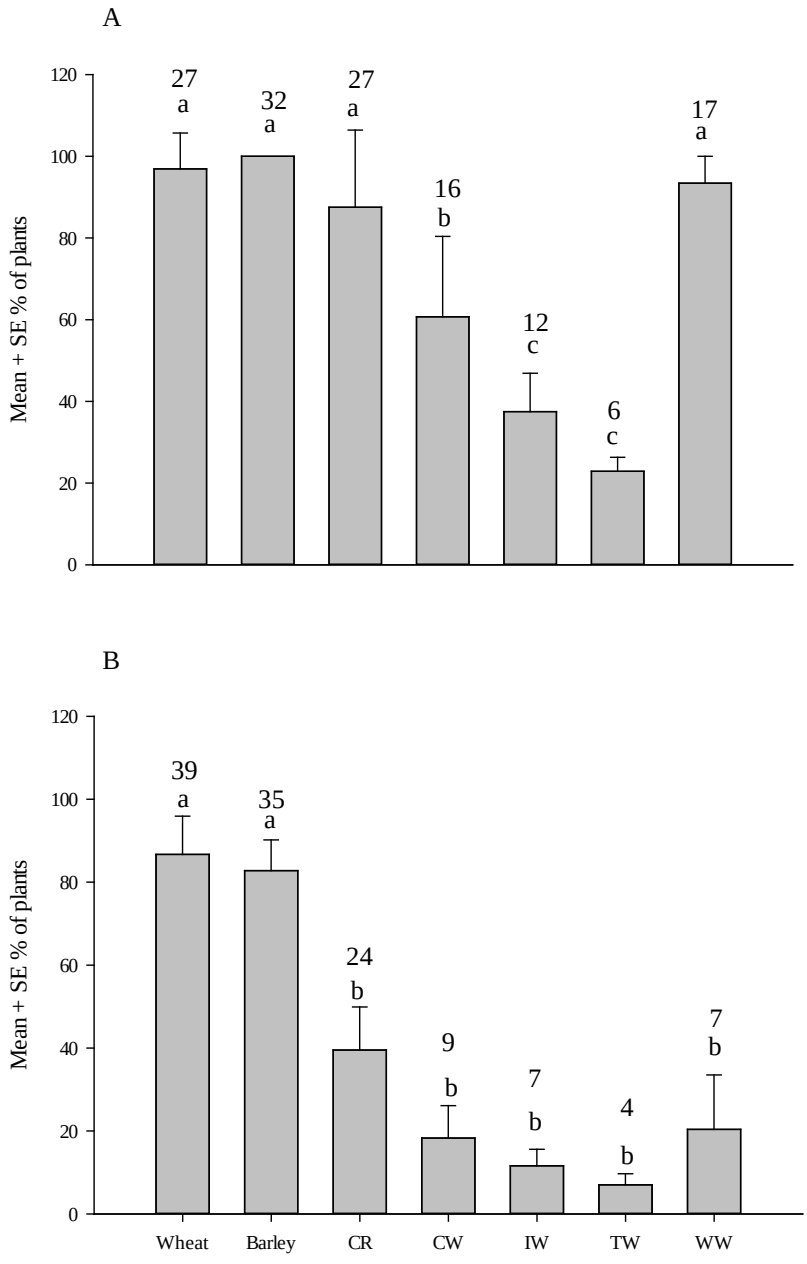

Figure 6. Percentage of plants per block of the seven grasses (A) on which living Hessian fly larvae were found and (B) that produced adult Hessian flies. At the top of each bar is shown the total number of plants with living larvae (A) or producing adults (B) within each figure. Means that do not share a letter are significantly different (means separation by Tukey-Kramer HSD test at P < 0.05).

tall wheatgrass (TW) (Tukey-Kramer HSD at $P < 0.05$). The percentage of plants that produced adults also varied across the seven grasses (Figure 6B, one-way ANOVA: $F = 18.63$, d.f. = 6, 45, $P < 0.0001$). Wheat and barley plants were more likely to produce adults than plants of the other grasses (Tukey-Kramer HSD at $P < 0.05$).

Survival of larvae at the feeding sites at nine days post-attack differed across the seven grass species when all plants were included, including plants with living larvae and plants without living larvae (Figure 7A, one-way ANOVA on square root transformed data: $F = 26.19$, d.f. = 6, 183, $P < 0.0001$). Survival on wheat, barley, Canada wildrye (CR), crested wheatgrass (CW), and western wheatgrass (WW) was higher than survival on intermediate wheatgrass (IW) and tall wheatgrass (TW) (Tukey-Kramer HSD at $P < 0.05$). When only plants with living larvae were considered, larval survival again varied across the seven grass species (Figure 7B, one-way ANOVA: $F = 6.18$, d.f. = 6, 129, $P < 0.0001$), with less survival on crested wheatgrass (CW), intermediate wheatgrass (IW), tall wheatgrass (TW) and western wheatgrass (WW) than on wheat (Tukey-Kramer HSD at $P < 0.05$).

Survival from egg to adult eclosion across the seven grasses was significantly different when all plants were included in the analysis, including both plants that produced adults and plants that failed to produce adults (Figure 8A, one-way ANOVA: $F = 10.69$, d.f. = 6, 307, $P <0.0001$). Survival on wheat was higher than on the other grasses. When plants not producing adults were excluded from the analysis, there was no difference in survival from egg to adult eclosion across the seven grasses (Figure 8B, Welch ANOVA: $F = 3.74$, d.f. = 6, 117, $P = 0.0002$). While wheat, barley, and Canada wildrye produced sufficient numbers of adults, the other four grasses produced very few (Table 8, Welch ANOVA: $F = 7.72$, d.f. = 6, 117, $P < 0.0001$). There was a positive relationship between the mean percentage of plants that produced

living larvae and the mean percentage of plants that produced adults (Figure 9, F = 10.08; d.f. = 1, 6; P = 0.02; t = 3.18, P = 0.02).

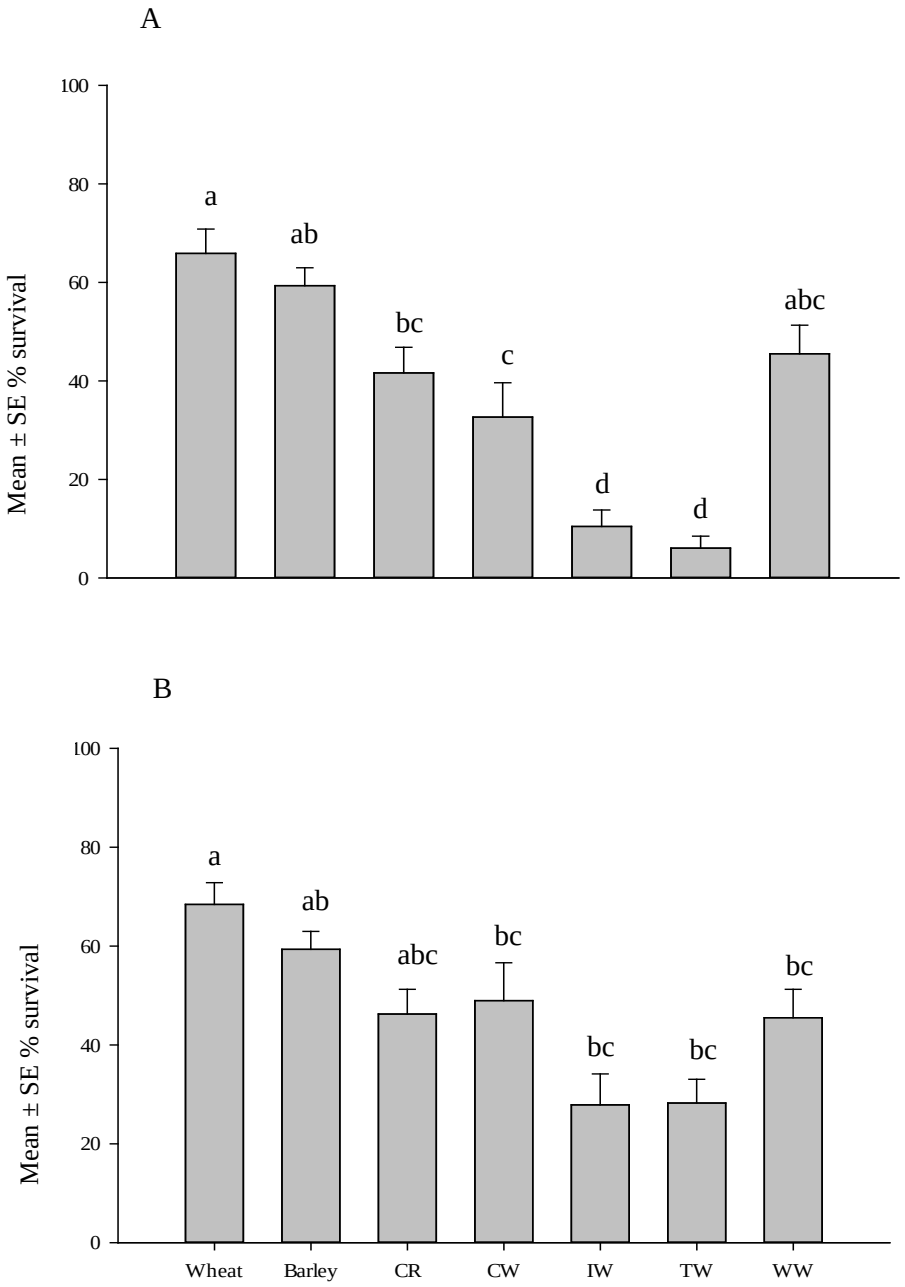

Figure 7. Survival of Hessian fly larvae during colonization of the seven grasses. In (A) percentage survival was calculated using all plants, including plants that did not have any living larvae. In (B) percentage survival was calculated using only the plants on which living larvae were found. Within each figure, means that do not share a letter are significantly different (one-way ANOVA on square root transformed data in (A) and one-way ANOVA in (B); means separation by Tukey-Kramer HSD test at P < 0.05).

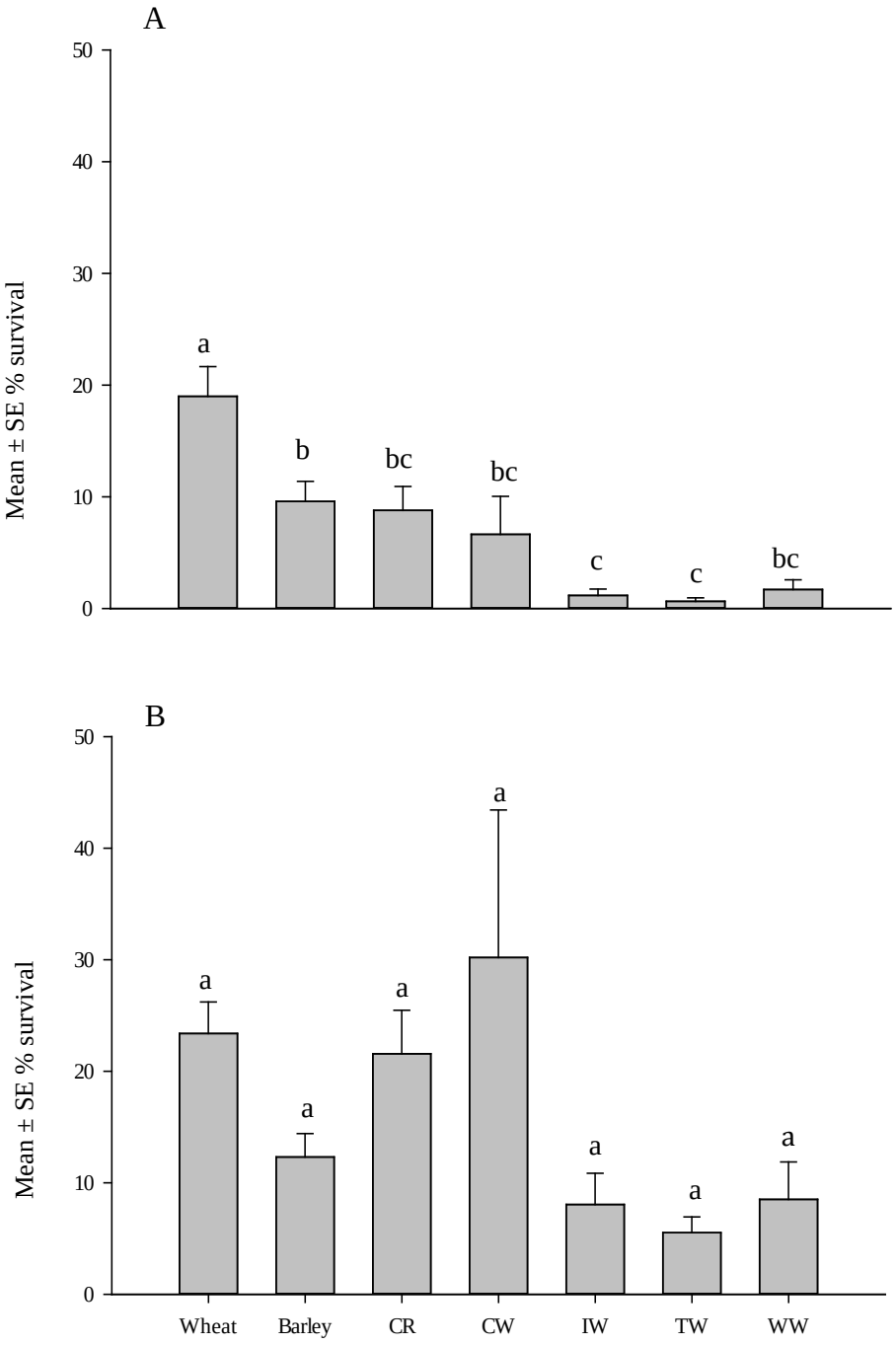

Figure 8. Survival of Hessian fly larvae from egg to adult eclosion on the seven grasses. In (A) percentage survival was calculated using all plants, including plants that did not produce any adults. In (B) percentage survival was calculated using only the plants that produced adults. Within each figure, means that do not share a letter are significantly different (one-way ANOVA and means separation by Tukey-Kramer HSD test at $P < 0.05$).

Table 8. Numbers of female and male Hessian fly adults that developed on seven grasses. 'Adults/plant' is based only on plants that produced adults. Within a column, means that do not share a letter are significantly different (ANOVA and means separation by Tukey-Kramer HSD test at P <0.05).

Species	# plants with adults	Adults/plant mean ± SE	Total # males	Total # females	Total # adults
Wheat	39	7.21 ± 0.95 a	126	154	280
Barley	35	3.29 ± 0.47 b	55	58	113
CR	22	2.95 ± 0.41 b	29	38	67
CW	9	2.00 ± 0.33 b	7	9	16
IW	7	1.57 ± 0.30 b	4	7	11
TW	5	1.40 ± 0.24 b	3	4	7
WW	7	1.71 ± 0.40 b	4	8	12

[1]Mean

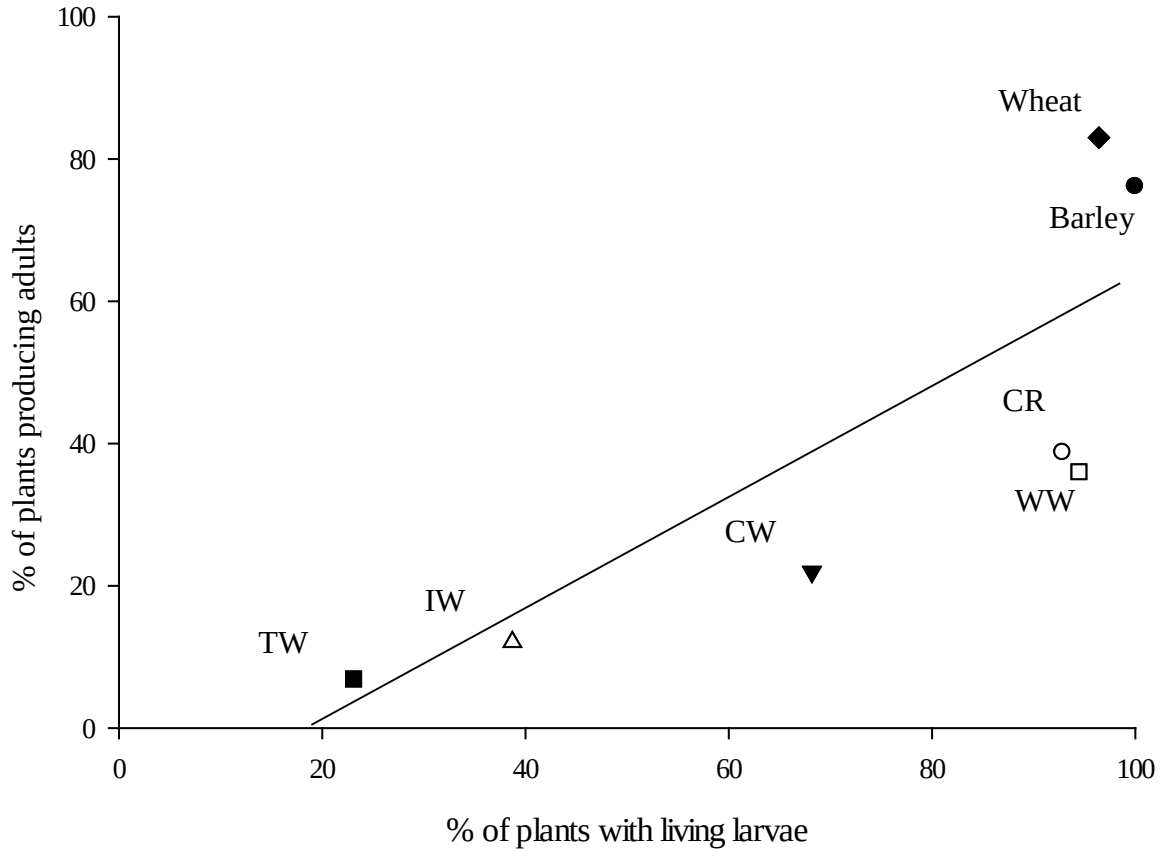

Figure 9. Relationship across seven grasses between the percentage of plants with living Hessian fly larvae and the percentage of plants that produced Hessian fly adults (Y = -18.35 + 0.78X, r^2 = 0.67).

For developmental time from egg deposition to adult eclosion, there were no differences across the seven grass species for both female (Figure 10A, Welch ANOVA: $F = 1.72$, d.f. = 6, 271, $P = 0.1673$) and male Hessian flies (Figure 10B, one-way ANOVA: $F = 0.95$, d.f. = 6, 221, $P = 0.46$). There was no relationship between the developmental times of females and males (Figure 11, $F = 0.0001$, d.f. = 1, 6, $P = 0.992$).

Wing lengths of adult females were different across the seven grass species (Figure 12A, one-way ANOVA: $F = 8.30$, d.f. = 6, 271, $P < 0.0001$), with wing lengths of females living on wheat and barley longer than those of females on crested wheatgrass (CW) (Tukey-Kramer HSD at $P < 0.05$). Wing lengths of males also varied across the seven grasses (Figure 12B, one-way ANOVA: $F = 6.24$, d.f. = 6, 221, $P < 0.0001$), with males on wheat and barley having longer wings than those on crested wheatgrass (CW) (Tukey-Kramer HSD at $P < 0.05$). There was a positive relationship between the average wing lengths of females and males (Figure 13, $F = 16.19$, d.f. = 1, 6, $P = 0.01$; $t = 4.02$, $P = 0.01$).

I used the data of Bergh et al. (1990) to estimate the reproductive potential of offspring produced on the seven grasses (Table 9). For females, the mean wing lengths I measured for the seven grasses fell within the range seen in Bergh et al. (1990) and therefore could be translated into potential fecundity of female offspring reared on the seven grasses (Table 9). For males, the mean wing lengths of males reared on crested wheatgrass (CW) and tall wheatgrass (TW) were below the range seen in Bergh et al. (1990) and therefore could not be estimated precisely. There was a positive relationship between egg to adult survival and the potential fecundity of females reared on the seven grasses (Figure 14, $F = 8.45$, d.f. = 1, 6, $P = 0.034$; $t = 2.91$, $P = 0.034$). By combining data on egg to adult survival (Figure 8A) and data on potential fecundity of female offspring reared on the seven grasses (Table 9), I was able to estimate how many offspring

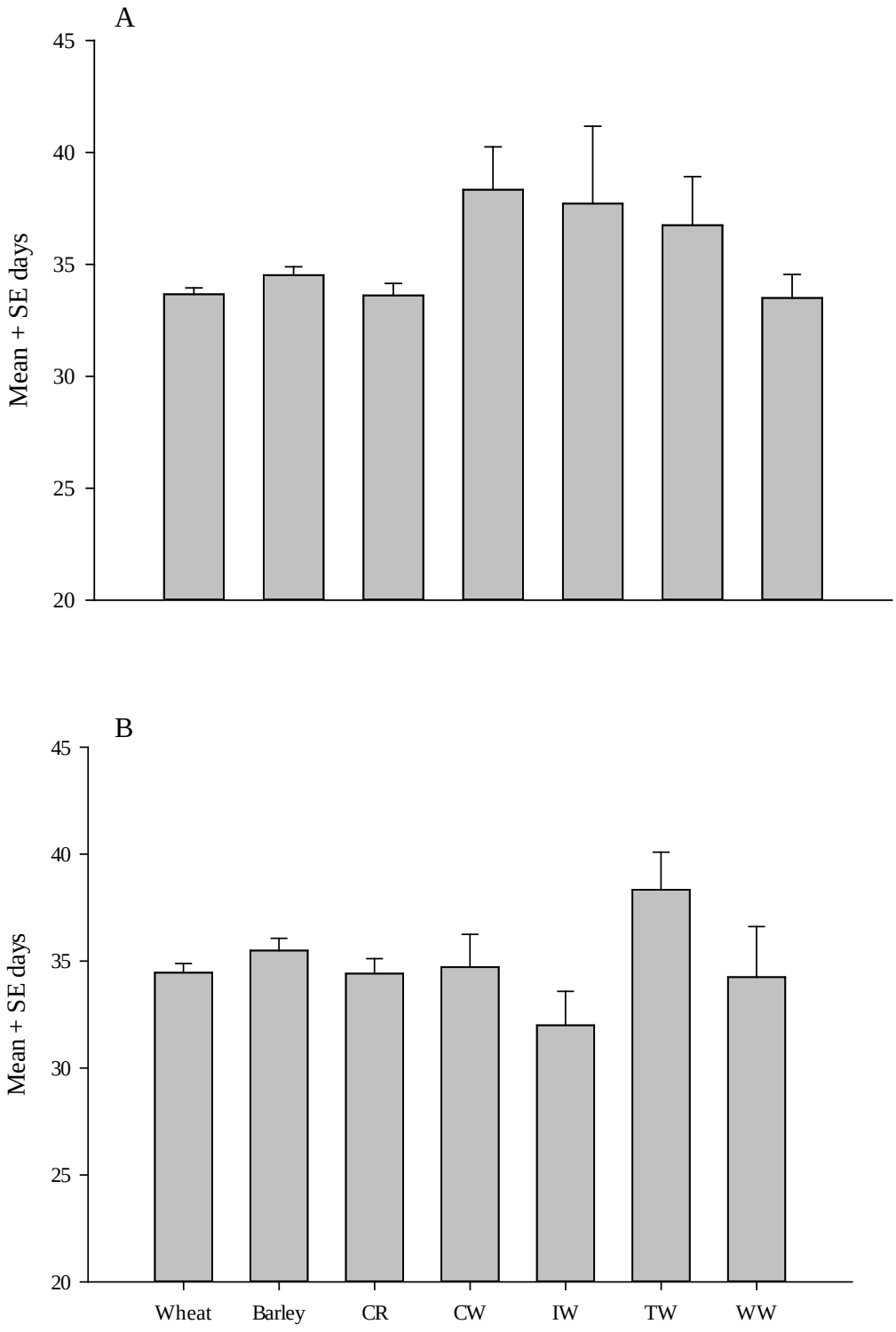

Figure 10. Developmental time of (A) female and (B) male Hessian flies from egg to adult eclosion on seven crop and prairie grasses. Developmental times did not differ across the seven grasses for either females or males (one-way ANOVA).

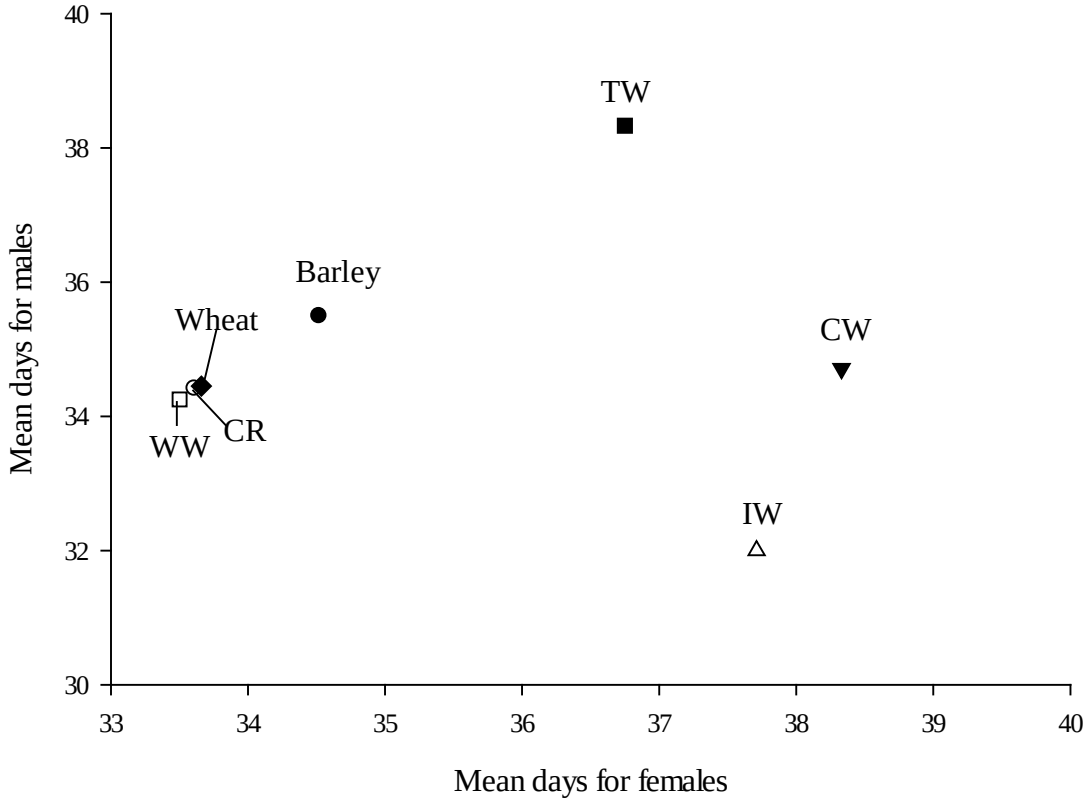

Figure 11. Relationship between developmental times of female and male Hessian flies that developed on the seven grasses.

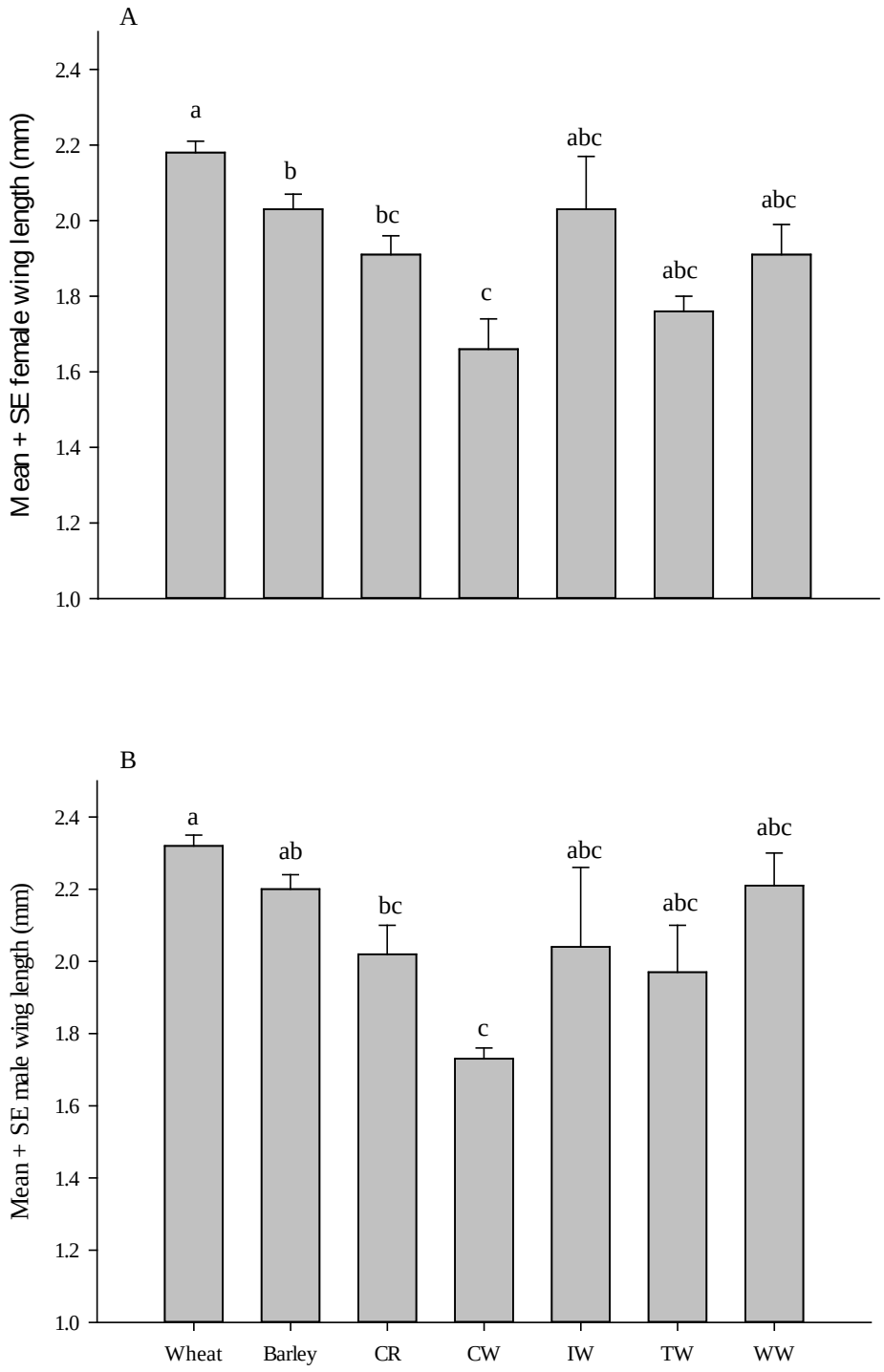

Figure 12. Wing length of (A) female and (B) male Hessian flies that developed on seven grasses. Within each figure, means that do not share a letter are significantly different (one-way ANOVA and means separation by Tukey-Kramer HSD test at $P < 0.05$).

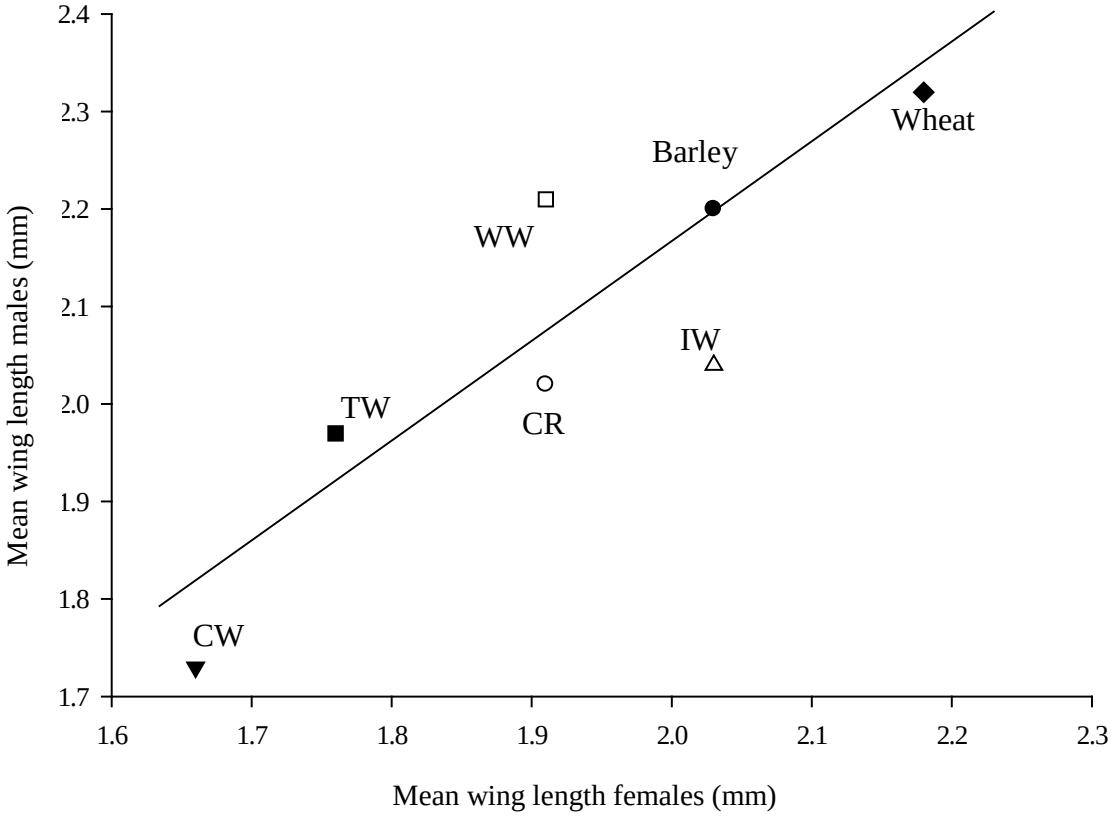

Figure 13. Relationship between sizes of female and male Hessian flies that developed on the seven grasses, using wing length to measure size ($Y = 0.2 + 0.97X$, $r^2 = 0.76$).

Table 9. Estimates of reproductive fitness of female and male Hessian flies that developed on seven different grasses. Data for translating adult wing length into eggs for females and number of matings for males came from Bergh et al. (1990).

Species	Mean female wing length (mm)	Estimated eggs	Mean male wing length (mm)	Estimated eggs fertilized
Wheat	2.18	104	2.32	2430
Barley	2.03	63	2.20	1719
CR	1.91	60	2.02	653
CW	1.66	40	1.73	645
IW	2.03	63	2.03	653
TW	1.76	40	1.97	650
WW	1.91	60	2.21	1719

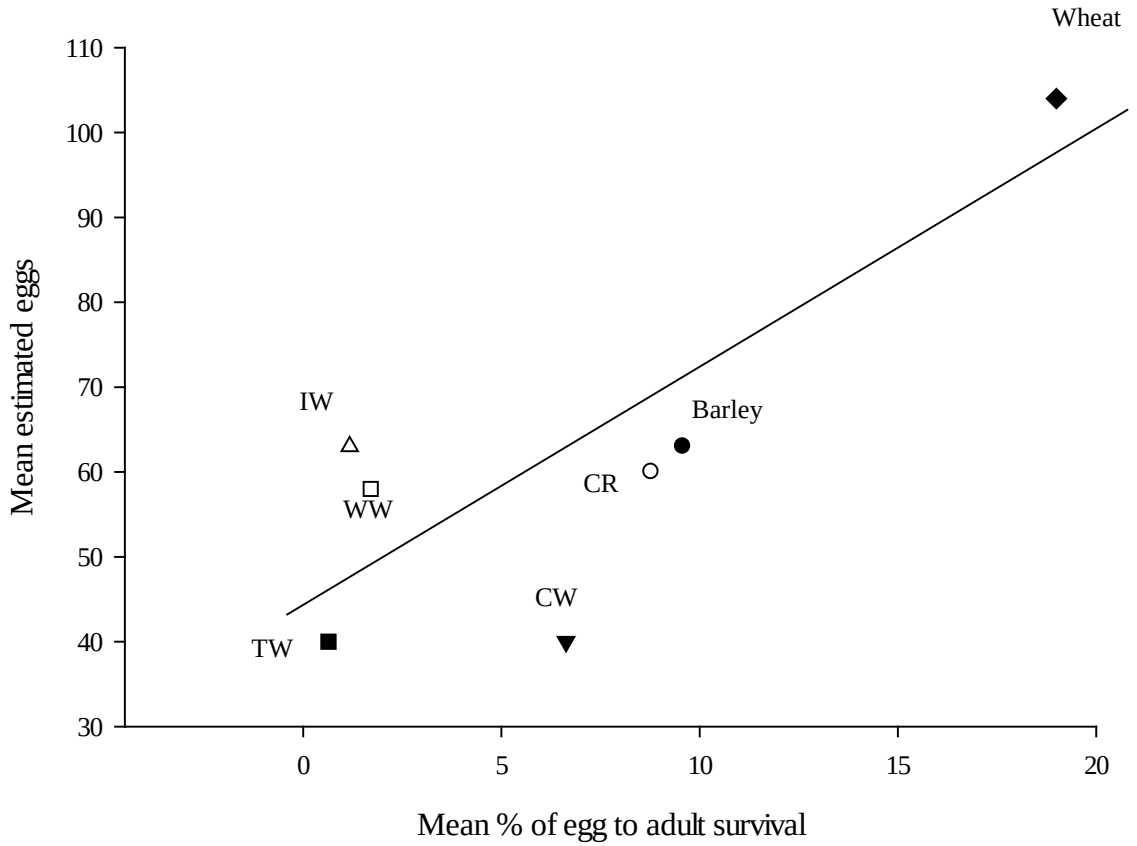

Figure 14. Relationship between Hessian fly survival on a grass species (Figure 8A) and potential fecundity of surviving females from Table 9 ($Y = 43.52 + 2.6X$, $r^2 = 0.63$).

would be produced by a female that lays all of her eggs on a particular grass. I used a fecundity of 250 eggs, which is the average fecundity of field-collected females (McConnell 1921). I also assumed that the female produces only female offspring. Unisexual progenies are a well-known feature of Hessian fly reproduction Stuart and Hatchett (1991). Figure 15 shows the predicted eggs produced by female offspring if all 250 of the eggs produced by the female are deposited on a particular grass species. Comparing these predicted eggs produced by offspring, i.e. offspring performance (Figure 15), with oviposition preference (Figure 4A), there was a positive relationship (Figure 16, $F = 8.66$, d.f. = 1, 6, $P = 0.032$; $t = 2.94$, $P = 0.032$).

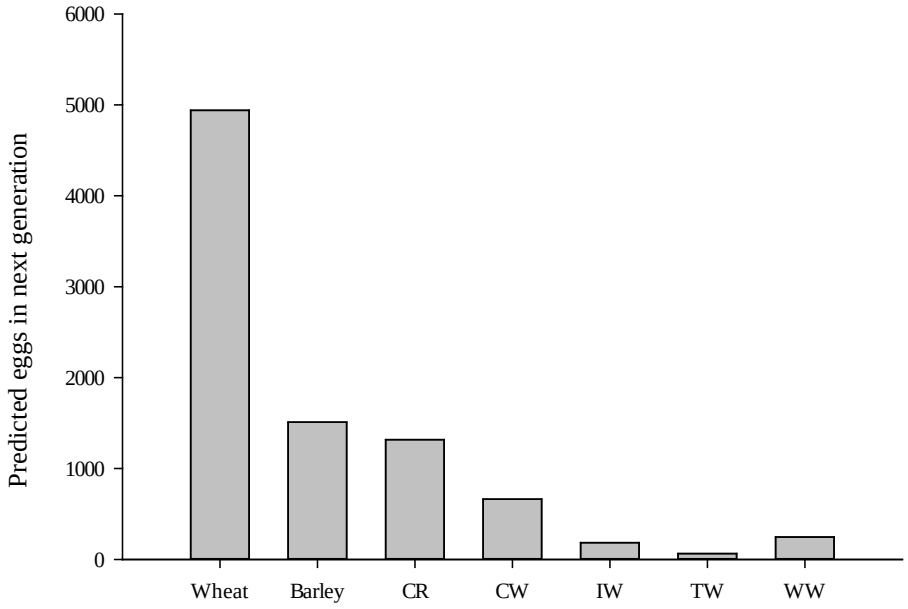

Figure 15. Fitness consequences of a Hessian fly female ovipositing all of her eggs on one of the seven grasses. Survival from egg to adult is taken from Figure 8A. Potential fecundity of female offspring is from Table 9.

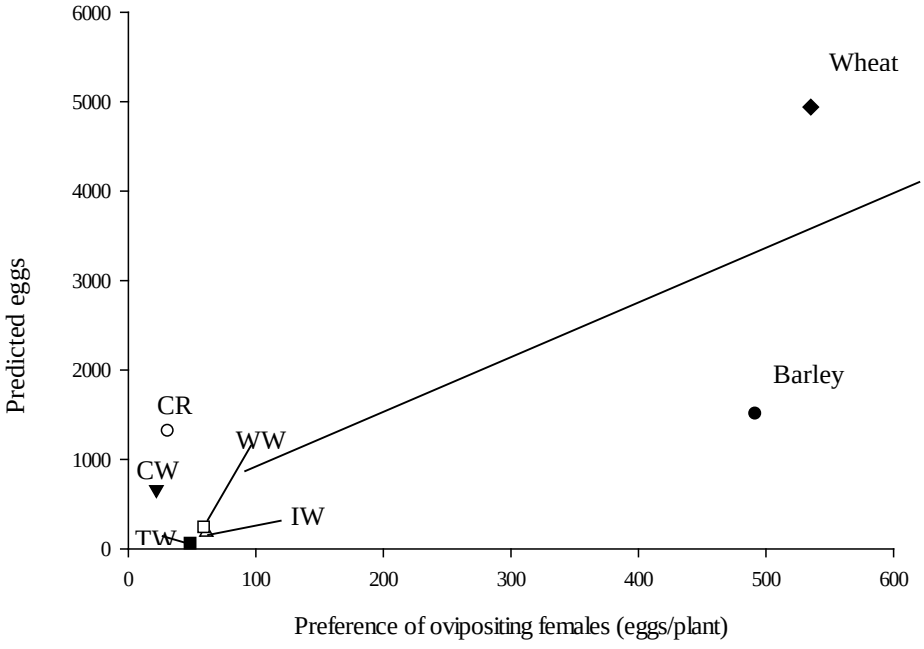

Figure 16. Relationship between Hessian fly preference and potential offspring fecundity (Y= 216.26 + 5.93X, $r^2 = 0.63$). Preference is from mean value shown in Figure 4A and fecundity is from predicted eggs in Figure 15.

Plant Responses to Attack

At both nine and 21 days post-attack (Figure 17), the third leaf of attacked plants was significantly shorter than that of non-attacked plants (Table 10) across all seven tested grasses.

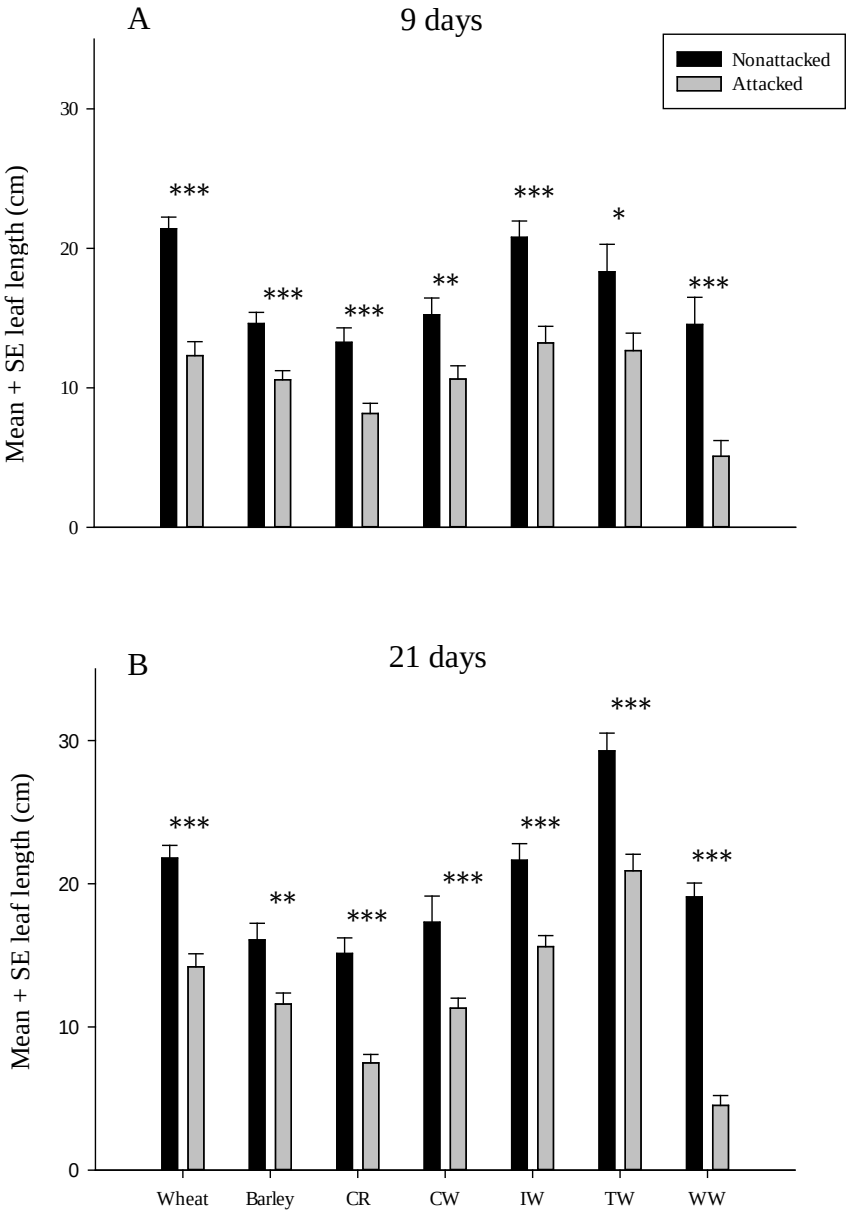

Figure 17. Growth of the third seedling leaf in response to Hessian fly larval attack across the seven grasses. (A) Nine days after larval attack began, when larvae have almost completed the second instar, and (B) twenty-one days after larval attack began, when larvae have stopped feeding and molted to the non-feeding third instar. Pairs of bars that are accompanied by asterisk(s) are significantly different at $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***) (one-way ANOVA or Welch ANOVA). Statistical comparisons are given in Table 10.

Table 10. Statistical tests for Figure 17 comparing growth responses of the third leaf to Hessian fly larval attack across the seven grasses.

Species	Effect of larval attack on leaf growth	
	9 day (Figure 17A)	21 day (Figure 17B)
Wheat	F = 48.94; d.f. = 1, 41; P < 0.0001[1]	F = 20.54; d.f. = 1, 59; P < 0.0001
Barley	F = 13.59; d.f. = 1, 45; P = 0.0006	F = 9.12; d.f. = 1, 60; P = 0.0037
CR	F = 16.14; d.f. = 1, 46; P < 0.0002	F = 35.14; d.f. = 1, 76; P < 0.001
CW	F = 8.90; d.f. = 1, 38; P = 0.0054	F = 16.84; d.f. = 1, 61; P < 0.0001
IW	F = 16.70; d.f. = 1, 45; P = 0.0002	F = 14.32; d.f. = 1, 72; P = 0.0003
TW	F = 6.4; d.f. = 1, 44; P = 0.015	F = 12.97; d.f. = 1, 74; P = 0.0006
WW	F = 20.26; d.f. = 1, 27; P < 0.0001	F = 105.7; d.f. = 1, 44; P < 0.0001

[1]Welch ANOVA was used for stats.

Analysis of the impact of attack on the 4th leaf differed because it was actively growing at the time of attack and therefore suffered greater impacts, including absence in attacked plants (Figure 18). At 9 days post-attack, attacked plants of wheat, barley, Canada wildrye (CR) and western wheatgrass (WW) were less likely to have produced a fourth leaf than non-attacked plants (Figure 18A, Table 11). At 21 days post-attack, attacked plants of all grasses except tall wheatgrass (TW) were less likely to have produced a fourth leaf than non-attacked plants (Figure 18B, Table 11). For the plants that had a fourth leaf at 9 days post-attack (Figure 19A), non-attacked plants of crested wheatgrass (CW) had a longer fourth leaf than attacked plants (Table 12). For plants that had a fourth leaf at 21 days post-attack (Figure 19B), non-attacked plants of wheat, barley and Canada wildrye (CR) had longer fourth leaf than attacked plants (Table 12).

Table 11. Statistical tests for Figure 18 comparing production of the fourth leaf across the seven grasses.

Species	Effect of Hessian fly larval attack on plants	
	9 days (Figure 18A)	21 days (Figure 18B)
Wheat	F = 15.83; d.f. = 1, 15; P = 0.0046[1]	F = 62.26; d.f. = 1, 15; P < 0.0001
Barley	F = 9.00; d.f. = 1, 15; P = 0.02[1]	F = 24.53; d.f. = 1, 15; P = 0.0002
CR	F = 7.12; d.f. = 1, 15; P =0.0184	F = 21.90; d.f. = 1, 15; P = 0.0004
CW	F = 0.05; d.f. = 1, 13; P = 0.8322	F = 21.39; d.f. = 1, 13; P = 0.0006
IW	F = 0.64; d.f. = 1, 15; P = 0.4384	F = 15.60; d.f. = 1, 15; P = 0.0017
TW	F = 1.00; d.f. = 1, 15; P = 0.334	F = 0.68; d.f. = 1, 15; P = 0.4248
WW	F = 1.23; d.f. = 1, 11; P = 0.2988[1]	F = 841.00; d.f. = 1, 11; P < 0.0001

[1]Welch ANOVA was used for statistical comparisons.

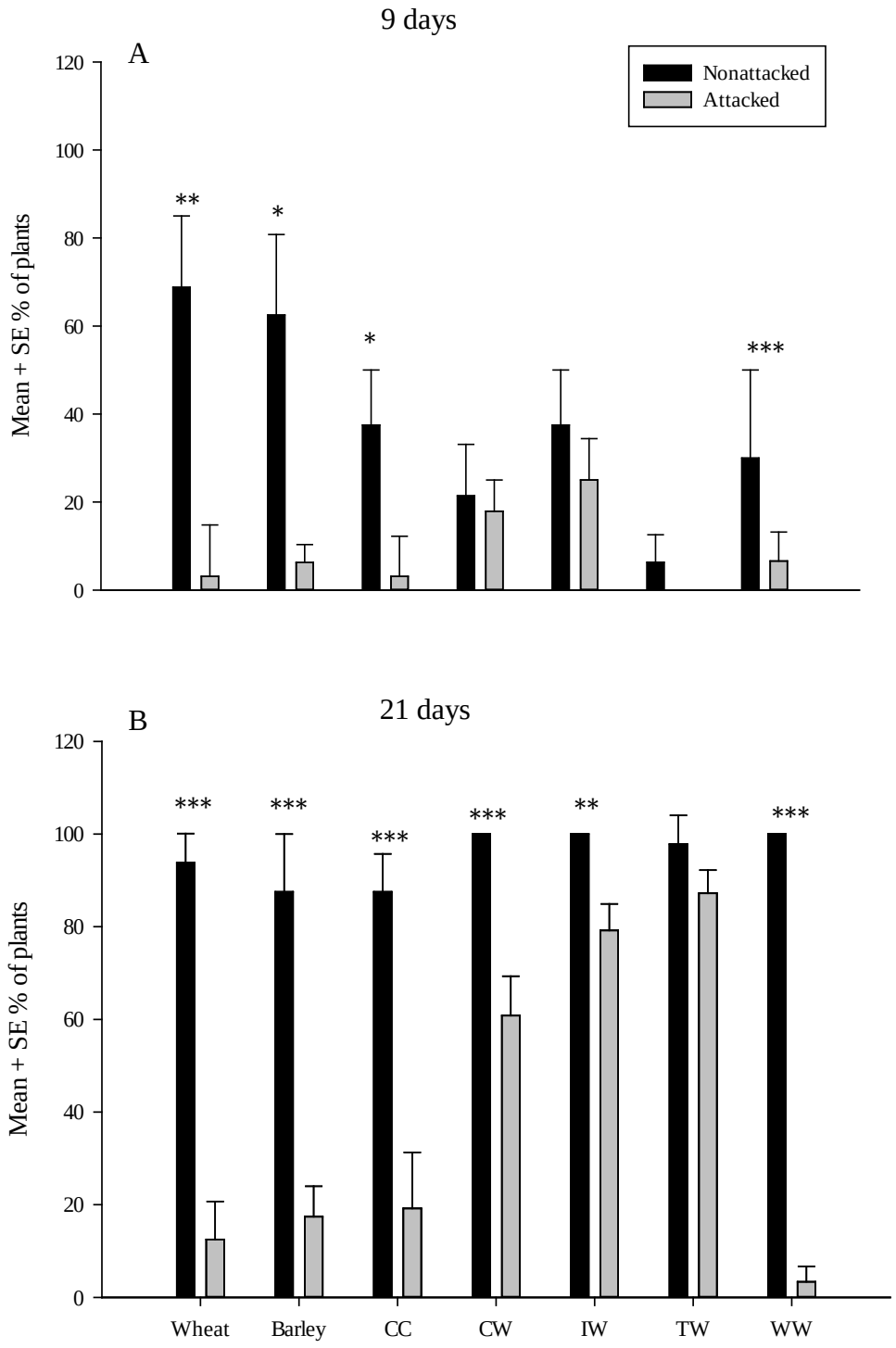

Figure 18. Percentages of plants of the seven grasses that had produced a fourth leaf (A) nine days or (B) twenty-one days after attack by Hessian fly larvae began. Pairs of bars that are accompanied by asterisk(s) are significantly different at $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***) (one-way ANOVA or Welch ANOVA). Statistical comparisons are given in Table 11.

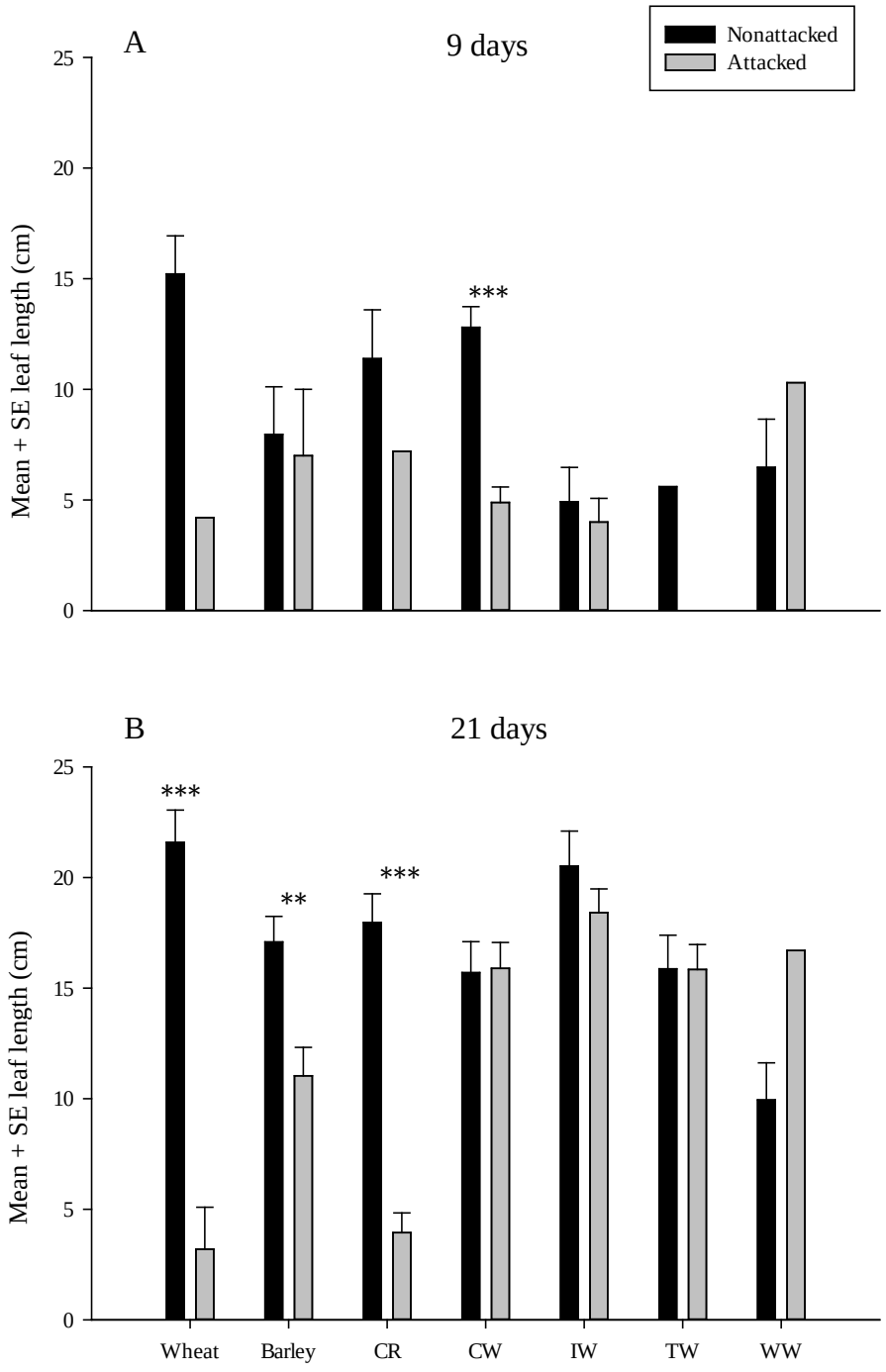

Figure 19. Growth of the fourth seedling leaf in response to Hessian fly larval attack across the seven grasses (A) nine days after larval attack began and (B) twenty-one days after larval attack was initiated. Only plants that had produced a fourth leaf were included in analyses. Pairs of bars that are accompanied by asterisk(s) are significantly different at $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***) (one-way ANOVA or Welch ANOVA). Statistical comparisons are shown in Table 12.

Table 12. Statistical tests for Figure 19 comparing the length of the fourth leaf across the seven grasses. Plants that did not produce a fourth leaf were excluded from analysis.

Species	Effect of Hessian fly attack on leaf growth	
	9 day (Figure 19A)	21 day (Figure 19B)
Wheat	--[1]	F = 65.95; d.f. = 1, 18; P < 0.0001
Barley	F = 0.39; d.f. = 1, 9; P = 0.85	F = 12.20; d.f. = 1, 23; P = 0.002
CR	--	F = 70.82; d.f. = 1, 23; P < 0.0001
CW	F = 46.94; d.f. = 1, 5; P = 0.001	F = 0.01; d.f. = 1, 41; P = 0.92
IW	F = 0.25; d.f. = 1, 11; P = 0.627	F = 1.05; d.f. = 1, 61; P = 0.31
TW	--[1]	F = 0.0002; d.f. = 1, 67; P = 0.99
WW	--[1]	--[1]

[1]No statistical comparisons due to small sample size.

The fifth leaf did not appear until after larval attack finished and therefore was measured at 21 days post-attack but not 9 days post-attack. At 21 days post-attack, attacked plants of wheat, barley and Canada wildrye (CR) were less likely to have produced a fifth leaf than non-attacked plants (Figure 20A, Table 13). For the plants that had produced a fifth leaf, none of the grasses showed an impact of larval attack on the length of the fifth leaf (Figure 20B, Table 13).

Table 13. Statistical results for Figure 20 comparing growth responses of the fifth leaf to Hessian fly larval attack across the seven grasses.

Species	Effect of larval attack	
	Plant percentage (Figure 20A)	Leaf growth (Figure 20B)
Wheat	F = 225; d.f. = 1, 15; P < 0.0001	--[2]
Barley	F = 88.44; d.f. = 1, 15; P < 0.0001	F = 0.60; d.f. = 1, 22; P = 0.448
CR	--[2]	--[2]
CW	F = 0.19; d.f. = 1, 13; P = 0.675	F = 0.0017; d.f. = 1, 25; P = 0.967
IW	F = 0.14; d.f. = 1, 15; P = 0.72[1]	F = 1.38; d.f. = 1, 32; P = 0.25
TW	F = 2.33; d.f. = 1, 15; P = 0.1489	--[2]
WW	--[2]	--[2]

[1]Welch ANOVA was used for statistics.
[2]No statistical comparisons due to small sample size.

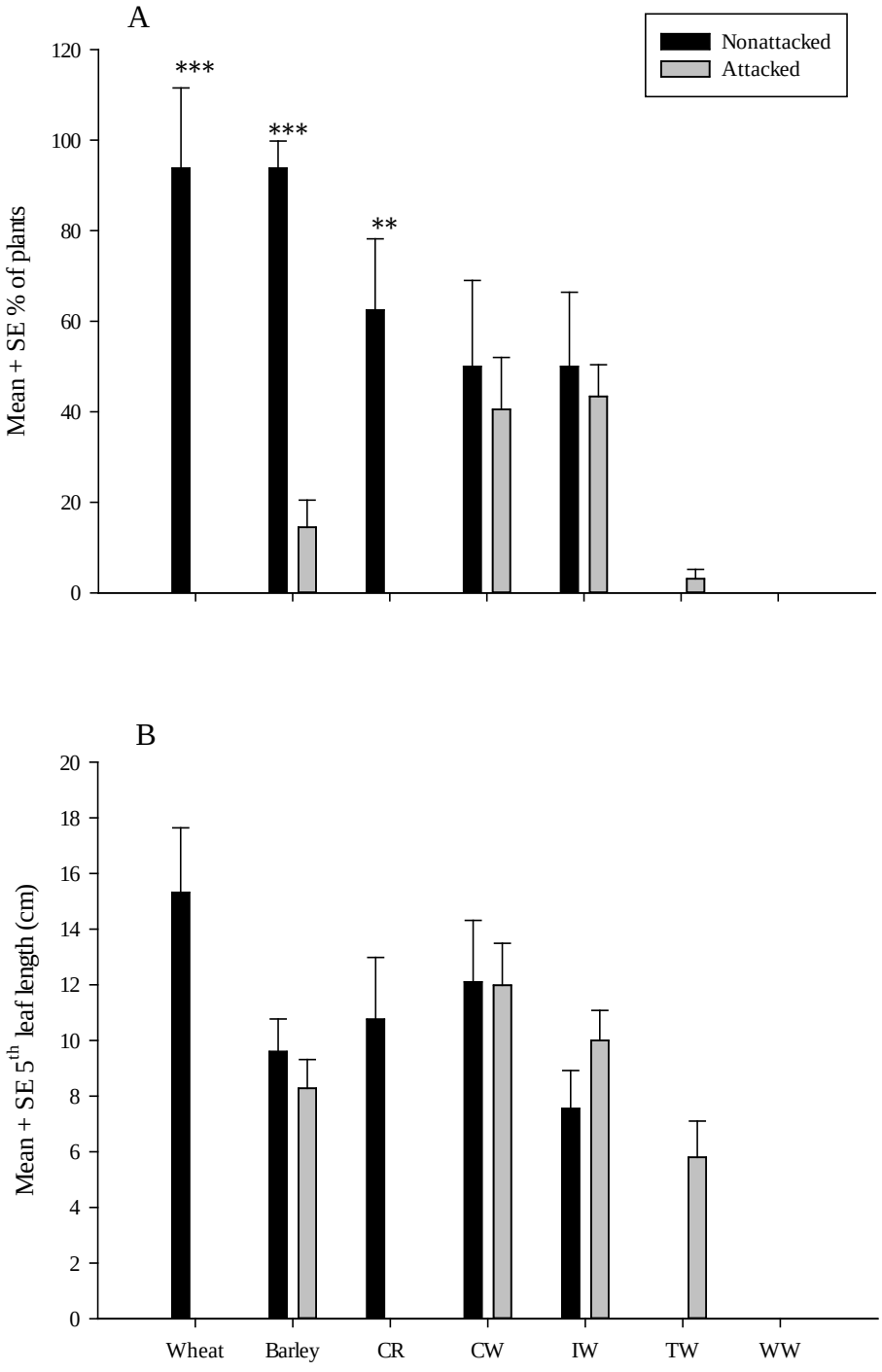

Figure 20. Growth of the fifth seedling leaf in response to Hessian fly larval attack across the seven grasses. In (A) is the percentage of plants that had produced a fifth leaf twenty-one days after larval attack was initiated. In (B) is the length of the fifth leaf for plants that had produced a fifth leaf by twenty-one days. Pairs of bars that are accompanied by asterisk(s) are significantly different at $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***) (one-way ANOVA or Welch ANOVA). Statistical comparisons are shown in Table 13.

Leaf lengths were totaled for leaves three, four and five for non-attacked and attacked plants to provide estimates of the growth loss suffered by plants attacked by Hessian fly larvae (Figure 21). At nine days post-attack, growth losses were greater for wheat, Canada wildrye (CR), and western wheatgrass (WW) than for crested wheatgrass (CW), intermediate wheatgrass (IW), and tall wheatgrass (TW) (Figure 21A, one-way ANOVA: $F = 7.44$, d.f. = 6, 191, $P < 0.0001$, Tukey-Kramer HSD at $P < 0.05$). At 21 days post-attack, growth losses again were greater for wheat, Canada wildrye (CR), and western wheatgrass (WW) than for crested wheatgrass (CW), intermediate wheatgrass (IW), and tall wheatgrass (TW) (Figure 21B, Welch ANOVA: $F = 54.56$, d.f. = 6, 350, $P < 0.0001$). The relationship between growth loss at nine days and 21 days is shown in Figure 22 ($F = 17.85$, d.f. = 1, 6, $P = 0.0083$; slope different from zero $t = 4.22$, $P = 0.0083$).

Discussion

The aim of my study was to expand knowledge of the host interactions of the Hessian fly. Interactions with crop and prairie grasses were investigated from two perspectives. The first was the perspective of the Hessian fly: how do prairie grasses compare as hosts to the well-studied crop hosts of the Hessian fly? I hypothesized that ovipositing females distinguish among crop and prairie grasses, measured as numbers of eggs oviposited on individual plants, and that the resulting ranking of the grasses is correlated with the performance of offspring reared on the grasses. The second perspective was that of the prairie grasses: is the Hessian fly a threat to growth or are seedlings able to resist attack? Here I hypothesized that, if Hessian fly larval colonization and growth is supported, seedling prairie grasses suffer growth losses that are similar to those that have been documented for susceptible wheat genotypes (Anderson and Harris 2006, 2008).

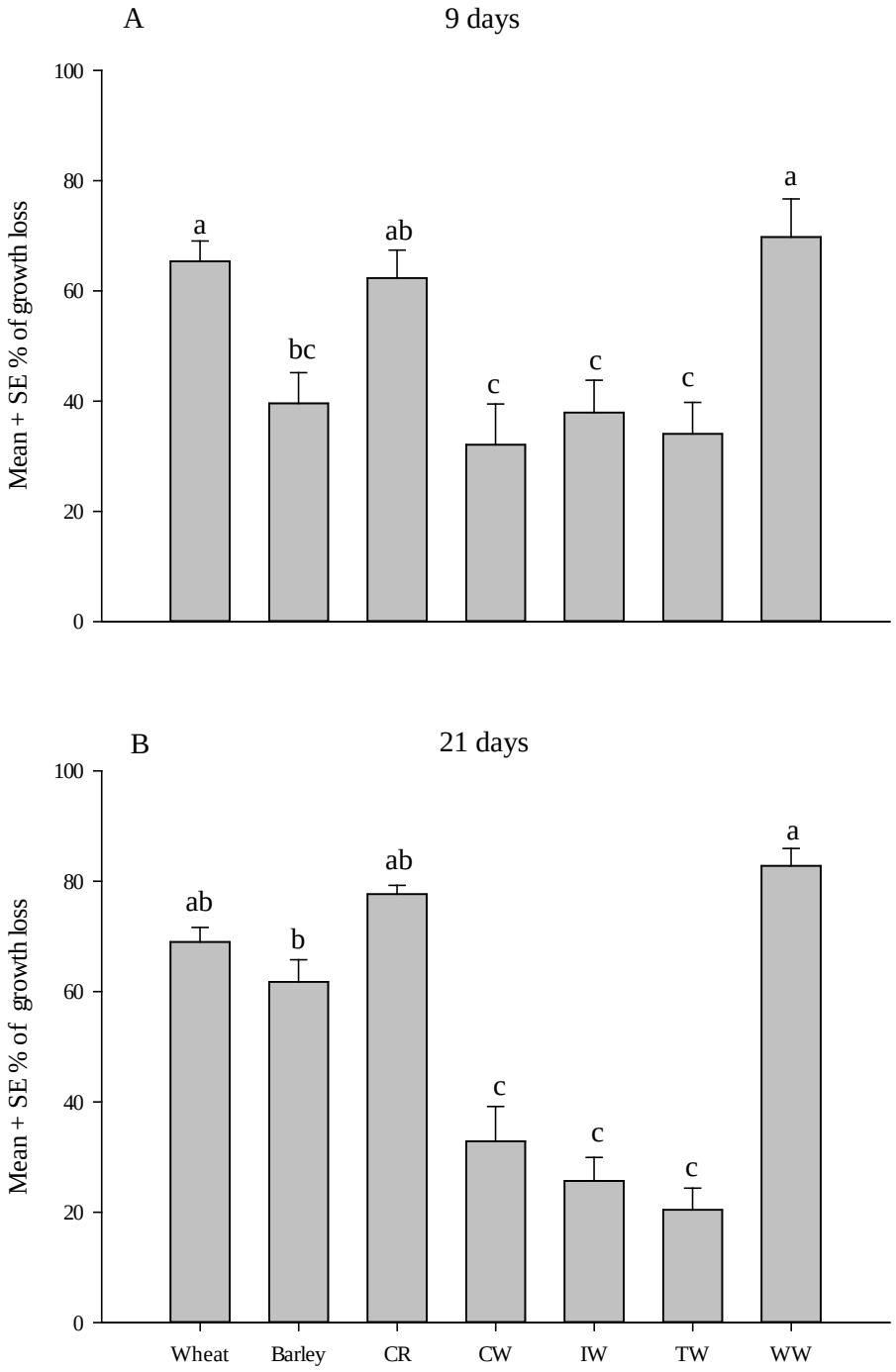

Figure 21. Growth loss across leaves three, four and five resulting from Hessian fly (A) nine days after larval attack was initiated and (B) twenty-one days after larval attack was initiated. Growth loss was calculated as (A) 1- [total length of leaves three and four of attacked plants/mean total length of leaves three and four of nonattacked plants] and (B) 1- [total length of leaves three, four and five of attacked plants/mean total length of leaves three, four and five of nonattacked plants]. Within each figure, means that do not share a letter are significantly different (one-way ANOVA and means separation by Tukey-Kramer HSD test at $P < 0.05$).

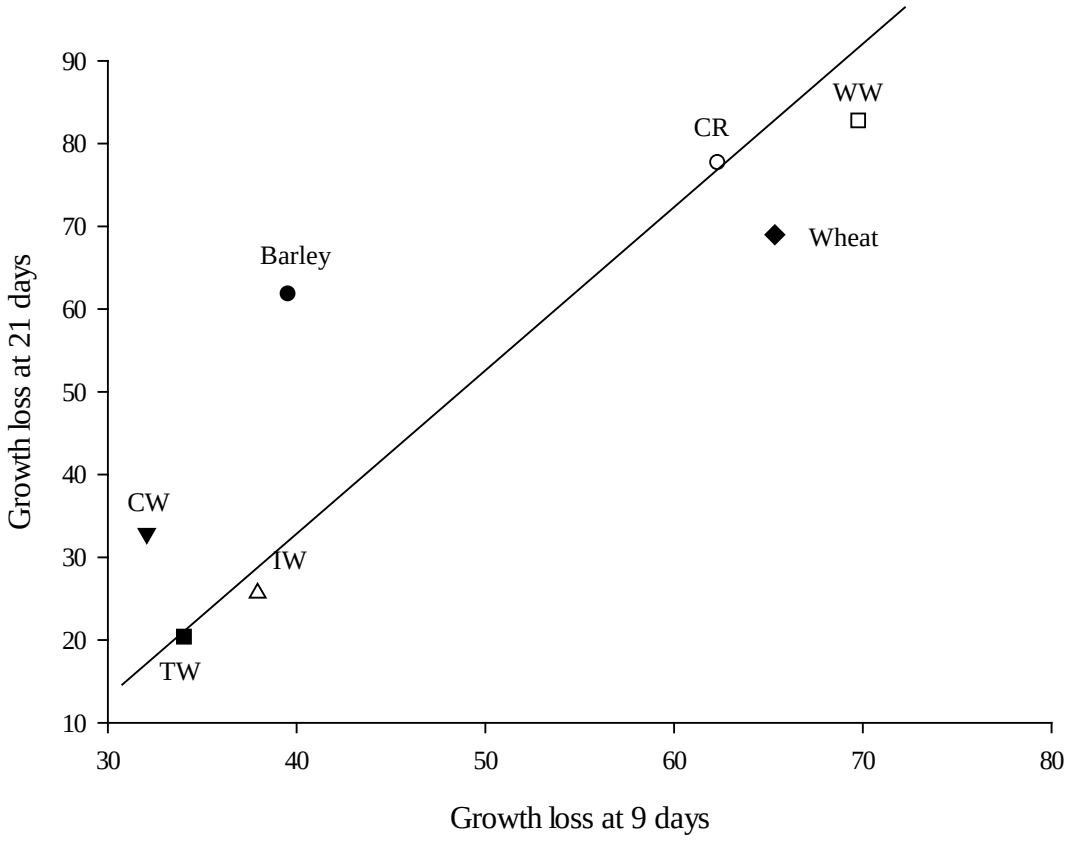

Figure 22. Relationship between plant growth loss from Hessian fly attack at nine and 21 days post-attack ($Y = -14.90 + 1.39X$, $r^2 = 0.78$). Growth loss at nine days is from Figure 21A, and growth loss at 21 days is from Figure 21B.

To measure oviposition responses, I first presented ten grass species to groups of mated Hessian fly females in a choice test. Three were crop species that represented a range of crop hosts, with wheat being the best-studied and most preferred host (Harris et al. 2003), barley being a somewhat less preferred host on which offspring do about half as well in terms of survival and growth as on wheat (Harris et al. 2001), and oat which receives few eggs from ovipositing females, except in no-choice tests over the lifetime of the ovipositing female (Harris and Rose 1989), and has never been found to support colonization (Harris et al. 2001). The other seven grass species (Table 4) were prairie grasses. All plants were at the two-leaf stage and were

presented to a group of females throughout the entire oviposition period (i.e. from the time eggs were first laid to the death of females, Harris and Rose 1991). In a second choice test, I removed the three crop species to determine whether Hessian fly females distinguish between the seven prairie grasses. A problem with choice tests is that they suffer from a lack of independence among treatments (Bruzzone and Corley 2011), because the response of the female to one treatment (i.e. one grass species) is likely to influence her response to the other treatments. If there had been more time, I would have also conducted no-choice tests to confirm the rankings that resulted from the choice tests (Harris and Rose 1989).

The two choice tests showed that Hessian fly females lay eggs on the seven prairie grasses (Figure 4) but not to the same degree as crop hosts. Thus, in the first oviposition choice test (Figure 4A), Hessian fly females placed many more eggs on wheat and barley. The statistically similar numbers of eggs received by wheat and barley contrasts with other studies that have shown that Hessian fly females lay twice as many eggs on wheat as on barley (Harris et al. 2001). In agreement with previous studies (Harris et al. 2001), oat received about 10% of the eggs seen on wheat and barley. It ranked alongside the prairie grasses, exceptions being the two brome grasses, meadow brome (MB) and smooth brome (SB), which received fewer eggs. The greater attractiveness of wheat and barley may be due in part to their greater surface area (Table 5). Ovipositing Hessian fly females respond to visual cues when orienting to potential hosts, with leaf area and vertical edges playing important roles (Harris et al. 1993).

In the second oviposition choice test with wheat, barley and oat removed as treatments (Figure 4B), Hessian fly females laid more eggs on intermediate wheatgrass (IW) and western wheatgrass (WW), which ranked higher than Canada wildrye (CR). Numbers of eggs on the other four prairie grasses remained small, especially for the brome grasses (Figure 4B). The

contrast between the two oviposition tests is interesting in that the pattern of choices in Figure 4B might not be predicted from the pattern of choices in Figure 4A, when the crop grasses were included. However, to make statistical comparisons between the two choice tests would require a different experimental design. The consistently lower ranking of the two brome grasses is expected given that insect herbivores are viewed as having a strong 'botanical instinct' (Schoonhoven et al. 2005). Brome grasses belong to the Bromeae, a different grass tribe than all other known hosts of the Hessian fly, which belong to the tribe Triticeae (Table 2). The preference of ovipositing females for particular sites within seedlings was, for the most part, consistent across crop and prairie grasses (Table 6), except that the preference for the youngest leaf was less evident for some of the prairie grasses, e.g. western wheatgrass, than for wheat.

Measures of offspring performance showed that the five prairie grasses can serve as hosts but are less suitable than wheat and barley. Percentages of prairie grass seedlings on which larvae were found nine days after larval attack was initiated (Figure 6A) and on which adults were produced (Figure 6B) were, for the most part, lower than on wheat and barley, particularly for the latter measure. Survival during the colonization stage was lower on the prairie grasses than on wheat even when plants having zero survival were excluded from the analysis (Figure 7A). In contrast, overall survival from egg to adult was lower on the prairie grasses than on wheat when plants having zero survival were included (Figure 8A) but not when plants having zero survival were excluded, when egg to adult survival was similar across the seven grass species.

Growth of larvae on the seven grasses is shown by the size of adults, measured by wing length (Figure 12). Here only two of the prairie grasses, i.e. Canada wildrye (CR) and crested wheatgrass (CW), produced smaller females (Figure 12A) and males (Figure 12B) than wheat.

Barley produced smaller females than wheat but not smaller males, and was only different from one of the prairie grasses, crested wheatgrass (CW). Impacts of the seven grass species were consistent for the size of females and males (Figure 13). Using winglength, I was able to estimate the reproductive success of adult males and females (Table 9) using the data of Bergh et al. (1990) for males, translating size into estimates of eggs that the male can fertilize, and Harris et al. (2001) for females, translating size into the eggs that the female develops and that are available for oviposition on plants. I combined these estimates of reproductive success with data on egg to adult survival (Figure 8A) to show the consequences of a female having 250 eggs (the average of field-collected Hessian fly females, McConnell 1921) laying all of those eggs on a particular grass. Here we can clearly see that the prairie grasses are poor hosts relative to wheat (Figure 15). Finally comparing the oviposition preferences of the Hessian fly to offspring performance (Figure 16) shows that there was a positive linear relationship between them, which indicated that among those seven species, female Hessian fly tends to select the grass species that can better support the development of its offspring.

Populations of phytophagous insect species that use a number of hosts can diverge when developmental times on hosts differ, causing assortative mating among adults developing on the same host. This is one mechanism that contributes to sympatric speciation (Schoonhoven et al. 2005). This does not appear to be the case for Hessian flies using different hosts. Developmental times from egg to adult were similar across the seven grasses (Figure 10). On the other hand, the divergence between male and female developmental times on several of the prairie grasses, e.g. 38 days for females and 32 days for males developing on intermediate wheatgrass (Figure 11), could cause problems for mating due to lack of synchronization. Hessian flies have a short adult lifespan of less than one day for males and females (Harris and Rose 1989). To some degree, a

possible lack of synchrony between males and females is mitigated by the temporal pattern of adult emergence within a population, which occurs over a period of two weeks (Bergh et al. 1990).

To a large degree, the five prairie grasses were similar to the susceptible wheat genotype in their responses to Hessian fly attack. All seven grasses were similar in suffering growth losses to the third leaf at nine and 21 days post-attack (Figure 17, Table 10). For the fourth leaf, the response pattern of crested wheatgrass, intermediate wheatgrass, and tall wheatgrass is interesting. When we look at the percentage of plants that had a fourth leaf at 21 days post-attack, nonattacked plants of crested wheatgrass and intermediate wheatgrass were more likely to have a fourth leaf than attacked plants (Figure 18B, Table 11), but for the plants that had a fourth leaf, the attacked plants did not suffer growth losses (Figure 19B, Table 12). Tall wheatgrass provided a different pattern. Here there was no difference between attacked and nonattacked plants for the percentage of plants with a fourth leaf (Figure 18B, Table 12) and there also was no difference in the growth of the fourth leaf (Figure 19B, Table 12). The idea that seedlings of tall wheatgrass are negatively impacted by larval attack but subsequently recover from attack is also supported by growth of the fifth leaf, which was greater for attacked versus non-attacked plants (Figure 20, Table 13). Canada wildrye and western wheatgrass fit the pattern of wheat in terms of impacts of larval attack (Anderson and Harris 2006, 2008). Thus, larval attack negatively impacted both the percentage of plants that had fourth leaf (Figure 18), the growth of the fourth leaf (Figure 19), and the percentage of plants with the fifth leaf and the growth of the fifth leaf (Figure 20). Based on my results I predict that crested wheatgrass, intermediate wheatgrass, and tall wheatgrass each contained a mixture of genotypes, some susceptible and therefore resembling susceptible wheat in their significant responses to Hessian fly attack, and

some resistant and therefore resembling resistant wheat in their minimal growth responses to Hessian fly attack (Anderson and Harris 2008).

Results from my experiments provide information that will be valuable for Hessian fly management. First, it demonstrates the need to control Hessian fly outside wheat fields. Wild grasses can serve as the hosts for the Hessian fly, which may influence the effectiveness of cultural control of the Hessian fly (Buntin and Chapin 1990). The Hessian fly can complete its life cycle on Canada wildrye, crested wheatgrass, intermediate wheatgrass, tall wheatgrass, and western wheatgrass. All of these grasses are important perennial grasses in the Northern Great Plains (USDA-NRCS 2012). During the Hessian fly's peak emergence, which occurs in late July and early August, spring wheat is too mature to serve as a host and winter wheat has not yet been sown (Anderson et al. 2012). If Hessian fly females emerge in wheat fields but find no oviposition sites, they will leave fields and travel distances to find hosts, as shown by mark-recapture studies (Withers et al. 1997). My research shows that if females encounter prairie grasses when no crop grasses are available, oviposition will occur and result in the plant serving as a host for Hessian fly larvae.

A second point is that the Hessian fly may threaten the growth and survival of prairie grasses. Larval survival on western wheatgrass was high and seedlings suffered major growth losses. This indicated that Hessian fly could have a negative impact on western wheatgrass, reducing its competitiveness in grassland plant communities. Western wheatgrass is used for erosion control in the northern Great Plains. Grasshoppers, ergot, and stem and leaf rust are the major problems for western wheatgrass (USDA-NRCS 2012) and it appears that the Hessian fly should be added to that list.

A third point is that crested wheatgrass, intermediate wheatgrass and tall wheatgrass might serve as the new sources of *resistance* genes to control the Hessian fly. By analyzing the relationships between offspring survival and the plant response, it was suspected that there were resistant genotypes in the above species, especially for intermediate wheatgrass which was preferred by female adults but exhibited variable responses (segregation) in offspring survival. Resistant genes to the Hessian fly have been found in *Triticum* spp., rye, and *Aegilops* spp. (Stebbins et al. 1982, Gill et al. 1986, Gill et al. 1991a, Gill et al. 1991b, Raupp et al. 1993, Ohm et al. 1995, Sardesai et al. 2005a, Sardesai et al. 2005b). *Resistance* genes are most amenable to transfer to adapted cultivars but are also viewed as vulnerable to 'defeat' resulting from pest adaptation (Bent and Mackey 2007). Having a greater number of resistance genes creates the possibility of stacking multiple genes in a single wheat cultivar, a strategy that might put the plant in a defense status that is beyond the evolutionary capacity of the Hessian fly to adapt. Many *resistance* genes effective against wheat pathogens have been found and identified in intermediate wheatgrass and tall wheatgrass, e.g. resistance to barley yellow dwarf virus, wheat borne disease, and stem and leaf rusts (Banks and Larkin 1995, Cai et al. 1996, McIntosh 1998, Sibikeeva et al. 2004, Li et al. 2005, Ayala-Navarrete et al. 2007). But scientists have not started to search for Hessian fly resistance genes in these species.

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