Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(5): 16-27

Received: 05-07-2017
Accepted: 06-08-2017

Devika Rani D
Department of Entomology, University of agricultural sciences, Bengaluru, Karnataka, India

Suneetha Pedada
Department of Entomology, University of agricultural sciences, Bengaluru, Karnataka, India

Ranvir Singh
Department of Entomology, University of agricultural sciences, Bengaluru, Karnataka, India

Correspondence
Devika Rani D
Department of Entomology, University of agricultural sciences, Bengaluru, Karnatala, India

Hessianfly: New insights to devbelop a gall

Devika Rani D, Suneetha Pedada and Ranvir Singh

Abstract
Gall midges constitute an important group of plant-parasitic insects. The Hessian fly (HF; Mayetiola destructor), the most investigated gall midge, was the first insect hypothesized to have a gene-for-gene interaction with its host plant, wheat (Triticum spp.). Recent investigations support that hypothesis. The minute larval mandibles appear to act in a manner that is analogous to nematode stylets and the haustoria of filamentous plant pathogens. Putative effector proteins are encoded by hundreds of genes and expressed in the HF larval salivary gland. Cultivar-specific resistance (R) genes mediate a highly localized plant reaction that prevents the survival of avirulent HF larvae. Fine-scale mapping of HF avirulence (Avr) genes provides further evidence of effector-triggered immunity (ETI) against HF in wheat. Taken together, these discoveries suggest that the HF, and other gall midges, may be considered biotrophic, or hemibiotrophic, plant pathogens, and they demonstrate the potential that the wheat-HF interaction has in the study of insect-induced plant gall formation.

Keywords: Cecidomyiidae, gene-for-gene interaction, resistance gene, avirulence gene, effector proteins, nutritive tissue

Introduction
The HF is one of the most economically important gall midges. It belongs to a genus containing 29 species whose larvae live on grasses. Its biology resembles that of the majority of cecidomyiids, but because its attack of wheat seedlings, and upto 50 larvae can survive on the same seedling (gall), it is the most amenable to propagation in the laboratory. HFs are easily reared and maintained at 17 to 24◦C in either the greenhouse or growth chamber. The life cycle is completed in approximately 28 days and consists of the egg, three larval instars, the pupa, and the adult. The pheromone that adult females use to attract mates has been identified. Females distribute their eggs (~200) on the upper surfaces of young wheat leaves. Only the first and second larval instars feed. After hatching, the first – instar larvae crawl to the base of the seedling, where they attempt to establish a feeding site. As described in greater detail below, first - instar larval modulation of plant development is critical to larval survival. Second - instar larvae are sessile and imbibe the liquids presented to them by the reprogrammed plant (the gall). Third-instar larvae and pupae develop within a puparium, which consists of the cuticle of the second - instar larva. This cuticle eventually hardens, sclerotizes, and becomes dark brown. Because of its appearance, this stage is commonly referred to as the flax seed. HF adults enclose from the puparia and live for only one to four days.

Ppest Status
The HF is present in North Africa, Europe, Western Asia, Central Asia, North America, and New Zealand. It can cause economic injury anywhere wheat is grown in the United States. The insect is often a greater problem in the southern United States because they rear typically more generations per year in the south (six to eight) than in the north, and there is no planting time when HF is dormant.

Recognition of insect by the plant
Recognition of herbivory-associated molecular patterns
Some interactions can be beneficial for the plant, as in the case of insect-mediated pollination or seed dispersion, and others are deleterious, as in the case of attack by herbivorous insects. To successfully combat aggressors, plants must be equipped with a sophisticated sensory system to perceive signals fast and efficiently from their environment and thereby detect potential enemies and subsequently translate and integrate such signals into appropriate

biochemical and physiological responses. Thus, upon attack, a number of reactions are detectable in plant cells, including changes in ion flux and protein phosphorylation, formation of reactive oxygen species and oxylipins, as well as initiation of various defense reactions in the host plant. Intriguing questions arising from these observations are how plants recognize the particular herbivores, what kinds of signals are involved, how such signals are perceived, and how they are converted into downstream signaling pathways involved in plant defense activation. Signal perception in the plant cell may rely on the presence of specific receptors for chemical signals or on general recognition processes based on localized tissue injuries. In principle, the feeding process combines two sites of the same coin: mechanical wounding of the infested tissue and introduction of oral secretions that are delivered from the feeding organism into the wounded tissue (i.e. the attacked plant is challenged by both a mechanical as well as a chemical stimulus). This Update introduces herbivore-derived metabolites, which represent serious candidates for signaling compounds; we will also discuss advances in herbivore recognition, namely, the perception of insect-derived signals by specific binding proteins. The properties of these binding proteins suggest their involvement in signal perception.

What can we learn from plant-pathogen interactions?

Signaling pathway studies revealed striking similarities in plant-pathogen and plant-herbivore interactions. For instance, typical elements of the battery of pathogen-induced plant responses that are also initiated after attempted herbivore infestation include enhanced ion fluxes across the plasma membrane and, very close to the biting zone, collapse of membrane integrity of challenged plant cells, activation of kinase cascades, and generation of reactive oxygen species. These localized defenses occur at the site of attack and are restricted to the treated leaf tissue. Moreover, generation of the phyto hormones jasmonic acid (JA), salicylic acid (SA), and ethylene, activation of defense-related genes, and synthesis of (volatile and non volatile) secondary compounds also can occur systemically. Thus, it seems tempting to speculate that the initial events concerning the recognition of pathogens or herbivores might be similar as well. Actually, wide spread usage of the term ‘‘elicitor’’ for signaling compounds that can stimulate herbivore-induced responses in plants has been borrowed from the pathogen field. Several models are conceivable to explain the basis of plant resistance against aggressors. Recognition of pathogens can be mediated either in a non host manner between a plant and pathogen species or in a gene- for gene interaction between a particular plant cultivar and a pathogen race. The high degree of specificity in the latter case is indicative of the co evolution of the antagonists, host, and pathogen, respectively. Non host disease resistance envisions a ligand-receptor-like interaction. In this case, plant immunity relies on the perception of chemical cues, general elicitors, which are present either constitutively in the pathogens or generated during pathogen invasion, by specific cell surface localized receptors. Such general elicitors are believed to merit the classification as pathogen-associated molecular patterns (PAMPs), whereas the corresponding receptors are referred to as pattern recognition receptors (PRRs). Recently, the more general term, microbe-associated molecular patterns (MAMPs), is preferred. MAMPs/PAMPs are perceived at low concentrations and act as inducers of defense reactions. MAMPs display diverse structures, including carbohydrates, (glyco)-proteins, lipids, peptides, and sterols. They are constituents of the outer layers of the pathogens, such as chitin fragments, b-glucan fragments, Pep-13, a peptide of the cell wall trans glutaminase of Phytophthora, or flg22, a peptide of the bacterial flagellin, but also an N-acetylated peptide of the bacterial elongation factor (EF)-Tu. For most of these MAMPs, the corresponding PRRs or binding sites have been genetically and biochemically identified: for b-glucans, the extracellular glucan-binding protein for chitin, the trans membrane LysM-containing receptor-like proteins, CEBiP and for flg22 and EF-Tu, the trans membrane Leu-rich repeat receptor-like kinases FLS2 and EFR respectively. At least the FLS2 receptor can be internalized followed by sub cellular redistribution and accumulation in endosomes. Typically, MAMP-triggered host responses are elicited fast and transiently, a fact that holds true also for responses elicited during herbivory. Moreover, because MAMPs are structurally rather diverse and the variety of induced responses in plant-pathogen and plant-herbivore interactions seems to have similar characteristics, it is tempting to propose that the basic processes in herbivore recognition in plants might be related to the recognition of pathogens. In the following, we will summarize our knowledge on both herbivore-derived elicitors and their corresponding binding sites in plants.

Herbivore- Associated molecular patterns as a new class of elicitors?

Oral secretions (OS) from feeding insects can contain herbivore- specific compounds with elicitor-like properties. According to the PAMP and MAMP classification, herbivore-derived elicitors will be denoted by herbivore-associated molecular patterns (HAMPs). This term will cover all herbivore-derived signaling compounds that might come into contact with the particular host plants during any stage of their life cycle and thereby elicit defense reactions. This can include, among others, components of OS, saliva, and oviposition fluid. Compared with the large number of MAMPs, up to now only few HAMPs have been isolated and their structures identified. Different proteins, such as Glc oxidase (Eichenseer *et al*., 1999) and alkaline phosphatase (Funk, 2001), have been shown to act as elicitor active compounds, and a b- glucosidase from larvae of the white cabbage butterfly (Pieris brassicae) triggered the release of volatiles from cabbage (*Brassica capitata*) leaves (Mattiacci *et al*., 1995).

Binding sites for the hamp volicitin

Recent progress in isolation of pure elicitors makes possible investigation of binding proteins, which might function as receptors in signal transduction pathways that ultimately activate defenses. However, up to now, such a study has been performed only for volicitin. Using a tritiated volicitin as radio ligand, in corn leaves the existence of a receptor-like binding site for volicitin has been demonstrated. The binding site is localized at the plasma membrane. It is heat and protease sensitive, and slightly (3-fold) inducible with methyl jasmonate, indicating its proteinaceous nature. The binding is of high affinity (Kd approximately 1.3 nM), saturable, reversible, and exhibits high ligand specificity. In particular, competition analysis revealed that the binding must be specific because the binding protein can discriminate between L- and D-volicitin enantiomers (i.e. conjugates with L- and D-Gln, respectively); only the biologically active L- volicitin competes with the radio ligand. All these parameters represent typical properties of classical receptors, which strongly suggests that, at least in corn, the volicitin effects are

mediated by receptor-connected processes. Next, the corresponding gene of the putative receptor needs to be cloned. In the future, the cognate receptors for the different HAMPs must be identified.

Wounding as signal?

Mechanical wounding of plant tissues is an inevitable consequence of herbivory. However, both intensity and extent of damage might be different and may vary with the mode of feeding (e.g. sucking [spider mites] or chewing [caterpillars]). The impact of injuries on the initiation of plant defense reactions has been underestimated for a long time. In almost all studies that investigated the effects of insect feeding, HAMPs, or both on the emission of, for example, volatiles, the corresponding control experiments have been performed using plants wounded by scratching, crushing, or puncturing leaves. Such types of mimicked herbivory resulted in induction of genes corresponding to different defense strategies but also in up-regulation of activities addressing changes in primary metabolism. Strikingly, induction of herbivory related volatile emission was not observed. Although it was obvious that wounding affected gene expression and physiological responses in planta, the question of whether or not such treatments are adequate to mimic insect feeding remained open. Herbivorous insects feed on leaves by continuously clipping off pieces of tissue, a long-lasting series of mechanical injuries. Thus, it is conceivable that plants are able to discriminate mechanical wounding that occurred only once and continuously sustained damage. This hypothesis has been proven by the deployment of Mec Worm, a computer-controlled mechanical caterpillar that simulates herbivory in a much more realistic mode. However, other electrical signals, such as variation potentials, might be involved as well. It will be interesting to figure out which kind of electrical signals are able to transport herbivory - induced information in principle and how such a signal can mediate specific information.

Establishment of feeding site

Changes in properties of wheat leaf cuticle during interactions with Hessian fly

The Hessian fly, *Mayetiola destructor* (Say), is a worldwide pest of bread (*Triticum aestivum* L. em Thell) and durum (*Triticum turgidum* L. var. *durum*) wheats. Within the USA it is known to cause extensive annual crop loss. During seedling infestation, adult female flies lay eggs on the leaf blade. Neonate (first-instar) larvae crawl down the lamina to protected areas between leaf sheaths and establish feeding sites on abaxial sheath surfaces. Although Hessian flies do not stimulate the formation of a macroscopic gall structure, as do related rice gall midges (*Orseolia oryzae* Wood-Mason), they are considered a gall midge due to the induction of nutritive tissue formation at feeding sites. The signals leading to plant abandonment of normal developmental patterns and transition to nutritive tissue formation largely remain unknown. It is thought that components of the larvae's saliva trigger changes in plant signaling pathways that lead to altered plant development. Nutritive tissues support larvae through two developmental instars, which are followed by a non-feeding third instar and pupation. The consequences of larval feeding on susceptible plants include stunted growth and frequently seedling death.

Host-plant-based genetic resistance is considered the most effective means of Hessian fly control. Generally, resistance is conferred by partially or completely dominant single genes and is manifested as larval antibiosis. To date, 32 resistance (*R*) genes (*H1* to *H32*) have been identified in wheat and related species. Incompatible gene-for-gene interactions occur when plants harbouring a specific single *R* gene are infested by larvae carrying the corresponding avirulence (*avr*) gene. Incompatible interactions result in the rapid elevation of plant defense gene mRNA levels, while avirulent larvae exhibit behaviours such as writhing and head-rearing as they encounter lectins and other potential feeding deterrents on the plant surface. Ultimately avirulent larvae are unable to establish feeding sites, due to alteration of the resistant plant's physiology, and die within 3–6 days after egg hatch.

The wheat–hessian fly interaction

Interactions between plants and their parasitic fungi, oomycetes, and nematodes suggest thatplant immunity has required each group of parasites to converge on a similar effector - based mechanism of attack.

The mechanics of attack, the presence of transcripts encoding putative effector proteins in the HF salivary gland, and the gene-for-gene manner in which wheat R genes provide HF resistance suggest that insect plant parasites use the same strategy. The physical interaction between wheat and HF begins when a neonate larva (460 μm long) emerges from an egg that was deposited on the upper surface of a young wheat leaf. The larva then uses the parallel venation of the leaf to guide its migration (1 cm h−1) down the leaf blade and enter the shelter that bundled leaf sheaths provide.

Within 1 to 2 cm of the base of the leaf, the larva attacks the still-expanding sheath epidermal cells of the abaxial surface of the adjacent, younger leaf. Six decades ago, Painter concluded that the first instar larva is incapable of physically rupturing plant cells, and recent investigations support that conclusion. First - instar HF larvae use paired, microscopic mandibles to penetrate into the cell wall. The tip of the mandible resembles the end of a hypodermic needle; it is grooved on the internal lateral surface, and this groove extends from the tip of the mandible internally into the basal hole. Salivary fluid is presumably delivered through the hole so that it travels down the groove and into the small punctures that have been observed in the cell walls of infested plants (53). The mandible blades extend into, or perhaps through, the epidermal cell wall but do not appear long enough to pierce the plasma membrane. They therefore appear to actinamanne that is analogousto a short stylet, or haustorium, that injects effectors into, or just below the cell wall without physically disturbing the plasma membrane. The cellular responses that follow this attack have been examined in both compatible and incompatible interactions.

The Compatible Interaction

To benefit its own growth, the successful larva alters the developmental pathways of wheat cells, severely compromising the growth of the plant. The epidermal and mesophyll sheath cells near the feeding site become the nutritive feeding cells that characterize all gall midge–induced galls. These have an enriched cytoplasm, an altered nucleus, and a thin cell wall that eventually breaks down to provide a liquid diet to the larva. Cell division and cell elongation cease, and chloroplasts accumulate. Outwardly, HF-infested susceptible wheat seedlings appear dark green and stunted. Although the seedling may compensate by tillering, the shoot apical meristem eventually dies. Plants that are attacked during stem elongation have similar symptoms, tend to lodge, and produce heads with less seed weight and fewer seeds.

Like other gall midges, the HF avoids inducing the production of plant volatiles that might attract parasitoids and predators. These symptoms are associated with altered patterns of plant gene transcription. Most upregulated genes of known function are involved in nutrient metabolism and transport. Some of these genes encode stress proteins (heat-shock proteins and components of the ubiquitin pathway), which may reflect the state of stress exerted by HF attack. Others encode transcription factors, which may be used to modulate plant development. The most interesting change is the coordinated up regulation of genes involved in primary metabolic pathways. These changes may reflect an elevated consumption of carbohydrates and an elevated synthesis of amino acids. This possibility is consistent with both the observation that the carbon-to-nitrogen ratiois. dramatically decreased at the feeding site and the requirements of an insect that lives on a food source that is normally nitrogen poor. Other up regulated genes may act to make nutrients more accessible for the growing larva. These include genes encoding a variety of nutrient transporters. Interestingly, the wheat gene Hfr-2, which encodes a cytolytic toxin-like protein with multiple agglutinin domains and a membrane-binding domain, is also up regulated. It is possible that this protein inserts into cell membranes and makes them more permeable. Not surprisingly, many plant defense genes are down regulated. These include genes encoding protease inhibitors, lectins, enzymes involved in secondary metabolite synthesis (O-methy ltransferases and chalcone synthases), enzymes involved in cell wall metabolism (xyloglucan endotransglycosylases and cellulose synthases, lipases and lipid transfer proteins, and class III peroxidases. Consistent with an inhibition of plant growth and a lowered demand for structural proteins, genes encoding various histones and a histone acetyl transferase are also strongly down regulate. In the HF, both the mandibles and the salivary glands display morphological changes that are correlated with changes in wheat morphology. Only four days after infestation by just a single larva, susceptible wheat seedlings are irreversibly compromised: Plant seedlings are stunted, plant defense genes are suppressed, metabolic pathways in the plant are reprogrammed, nutrient tissue forms, and the cell walls near the feeding site become thin and permeable. During this period, the larva remains a first instar, its mandibles are sharp, and the basal cells of the salivary gland are fully developed. After the plant has been irreversibly transformed into a permeable nutrient sin, the larva molts into a second instar, its mandibles are blunt, and the basal salivary gland cells begin to decay. Thus, the first instar larval stage is critical to gall formation and insect survival, and the first – instar larval salivary gland is the most obvious source of the factors the insect uses to modulate plant development.

Putative Effector Proteins Within the first - instar salivary gland, more than 50% of all transcripts encode proteins containing a secretion signal. Lessthan 5% of these encode proteins with sequence similarity to known proteins; these include proteases and protease inhibitors which are also expressed in the larval gut, and lipase-like proteins. The remaining signal peptide–encoding transcripts encode putative effector proteins called secreted salivary gland proteins (SSGPs). SSGPs lack sequence similarity to any other known proteins. Hundreds of SSGP-encoding transcripts have been classified into families and super families on the basis of sequence similarities. The majority of these encode small (50to 250-residue) proteins. Genomic analyses of a few SSGP families found that most of the related transcripts are non allelic; that family members are often clustered within small chromosomal segments; and that within these segments, the genes appear to be experiencing strong positive selection and functional adaptation.

Role of genes in susceptibility of host

Hessian fly avirulence genes

The existence of HF R genes in wheat and putative effector - encoding genes in the HF supports the hypothesis that the same ETI that underlies gene-for-gene interactions between plants and plant pathogens also underlies wheat-HF incompatible interactions. To test that hypothesis further, genetic analyses have been performed to determine if avirulence can be attributed to effector-encoding Avr genes. Hatchett & Gallun began these investigations, showing that virulence (the ability of HF larvae to survive on and stunt wheat seedlings) to the R gene H3 and virulence to the coordinated R gene pair H7, H8 are conditioned by independent, simply inherited, recessive genetic factors. Within a few years, Gallun's group had obtained evidence of the first X-linked HF Avr gene and extended the gene-for-gene association to four R genes in wheat (H3, H5, H6, and H7, H8). With the discovery that virulence to H9 and virulence to H13 were clearly X-linked, the convention of placing a small v (for recessive virulence to) in front of the R gene name was adopted in naming HF Avr genes (vH9 and vH13) (41, 127). The adoption of polymerase chain reaction (PCR)-based methods in these investigations permitted greater resolution in testing the gene-for-gene hypothesis, and the current ability to resolve Avr gene positions on the FPC-based physical map leaves little question regarding the hypothesis's veracity. To date, six HF Avr genes (vH5, vH6, vH9, vH13, vH24, and vHdic) have been mapped within chromosome segments spanning less than 600 kb. These solution of the genes that are near telomeres (vH9, vH13, and vH24), where recombination rates are greatest, is even better.

Polyamines role in wheat plant susceptability

Polyamines are ubiquitous, low-molecular-weight aliphatic poly cations that play a vital role in regulating gene expression, signal transduction, ion-channel function, DNA and protein synthesis as well as cell proliferation and differentiation. They scavenge reactive oxygen species thereby protecting DNA, proteins, and lipids from oxidative damage. In plants, the most common polyamines are diamine putrescine, triamine spermidine, and tetramine spermine. They occur either in free form or as conjugates bound to phenolic acids and low molecular weight compounds. Due to their positive charge, polyamines interact with negatively charged macromolecules such as proteins and nucleic acids leading to the stabilization of these molecules under stress conditions.

In plants, the first step in polyamine biosynthesis is the formation of putrescine from either ornithine or arginine. Ornithine is converted directly into putrescine by ornithine decarboxylase (ODC). Arginine can be converted into ornithine by arginase, or can take a longer route whereby it is converted to agmatine by arginine decarboxylase (ADC), then to *n*-carbamoyl putrescine by agmatine deiminase and finally into putrescine by *n*-carbamoyl putrescine amido hydrolase. Putrescine subsequently receives an amino propyl moiety from decarboxylated S-adenosyl methionine (SAMDC) via spermidine synthase (SPDS) to produce spermidine; and spermine is then generated by a second aminopropyl transfer by spermine synthase (SPMS).

Antioxidant defense response in a galling insect

Herbivorous insect species are constantly challenged with reactive oxygen species (ROS) generated from endogenous and exogenous sources. ROS produced with in insects because of stress and pro oxidant allele chemicals produced by host plants in response to herbivory require a complex mode of antioxidant defense during insect/plant interactions. Some insect herbivores have a midgut based defense against the suite of ROS encountered. Because the Hessianfly (*Mayetiola destructor*) is the major insect pest of wheat worldwide, and an emerging model for all gall midges, we investigated its antioxidant responses during interaction with its host plant. Quantitative data for two phosphor lipid glutathione peroxidases (MdesPHGPX-1 and MdesPHGPX-2), two catalases (MdesCAT-1 and MdesCAT-2), and two superoxide dismutases (MdesSOD-1 and MdesSOD-2) revealed high levels of all of the mRNAs in the midgut of larvae on susceptible wheat (compatible interaction). During development of the Hessian fly on susceptible wheat, a differential expression pattern was observed for all six genes. Analysis of larvae on resistant wheat (incompatible interaction) compared with larvae on susceptible wheat showed increased levels of mRNAs in larvae on resistant wheat for all of the antioxidant genes except MdesSOD-1 and MdesSOD-2. We postulate that the increased mRNA levels of MdesPHGPX-1, MdesPHGPX-2, MdesCAT-1, and MdesCAT-2 reflect responses to ROS encountered by larvae while feeding on resistant wheat seedlings and/or ROS generated endogenously in larvae because of stress/ starvation. These results provide an opportunity to understand the cooperative antioxidant defense responses in the Hessian fly/ wheat interaction and may be applicable to other insect/plant interactions.

Nutritional sink formation in galls

Nutritional resource manipulation is typical of insect-plant gall interactions. Galls act as physiological sinks providing insects with essential nutrients needed for their growth and development. Insect galls, galled (diseased) leaves and un-galled (healthy) leaves of WHAET infested by the gall making insect were collected to study the different changes resulting from the biotic stress caused by insect feeding. The first instar nymph initiates gall formation during the feeding stage by secreting saliva rich in proteins and lytic enzymes. This leads to localized cancerous growth causing mobilization of nutrients such as reducing sugars (RS), total soluble sugars (TSS), starch, free amino acids (FAA), proline and protein to the gall from the un-galled region of the leaf. A corresponding decrease of these nutrients was noted in the latter. Higher levels of total phenols (TP) and ortho - dihydric phenols (OP) were observed in galls when compared to the galled leaf. In addition, activities of amylase (AMY) and invertase (INV) were found to be higher in galls than in the galled leaf.

The galls and leaves (both galled and healthy) were sampled. Chilled galls were quickly dissected, and the gall forming aphids were removed from the galls. The frozen samples were homogenized with pre-chilled 50 mM sodium phosphate buffer (pH 7.0) containing 5 mM β mercaptoethanol and 1 mM EDTA using pestle and mortar. The homogenate was centrifuged at 3000 rpm for 15 min at 4 ºC. The supernatant was used to assay all parameters under consideration.

Reducing sugars (RS)

Reducing sugars were estimated according to Miller (1972). 500 mg of plant material was treated with 10.0 ml of 80 % ethyl alcohol. 3.0 ml of DNS (3, 5-dinitro-salicylic acid) reagent was added to 3.0 ml of the alcoholic extract. The mixture was heated for 5 min in a boiling water bath. After colour development, 1.0 ml of 40 % Rochelle salt was added. The tubes were cooled under running tap water. Absorbance was recorded using a spectrophotometer at 520 nm. The amount of reducing sugar was calculated using a standard curve prepared from glucose. The quantity of reducing sugar was expressed as mg g-1 fresh weight of tissue.

Total soluble sugars (TSS)

The amount of total soluble sugars was estimated by a Phenol sulphuric acid reagent method (Dubois *et al*. 1951). 500 mg of each plant material was homogenized with 10.0 ml of 80 % ethanol. Each sample was centrifuged at 2000 rpm for 20 min. 1.0 ml of 5 % phenol solution was added to 1.0 ml of the supernatant. Then 5.0 ml of 96 % sulphuric acid was added rapidly. Each tube was gently agitated and allowed to stand in a water bath at 30 ºC for 20 min. The optical density (OD) was measured in a spectrophotometer at 490 nm against the blank. A standard curve was prepared by using known concentration of glucose. The quantity of TSS was expressed as mg g-1 fresh weight of tissue.

Starch

Estimation of starch was carried out by the method of Mc Cready *et al*. (1950). The residual mass obtained after the extraction of soluble sugar was suspended in 5.0 ml of water and subsequently 6.5 ml of 52 % perchloric acid was added to the residue, the contents were centrifuged for 20 min at 2000 rpm. The supernatant was decanted and collected and the whole procedure was repeated thrice. The supernatant of each step were then poured into a standard flask and the total volume was made up to 100 ml with distilled water. The mixture was then filtered through What man filter paper (No. 42). 1.0 ml aliquot of this filtrate was analyzed for starch content following the same procedure as that of total soluble sugar. Quantity of starch was calculated in terms of glucose equivalent and factor 0.9 was used to convert the values of glucose to starch. The quantity of starch was expressed in terms of mg g-1 fresh wt. of tissue.

Pathogenesis-Related (PR) Proteins

Higher plants have a broad range of mechanisms to protect themselves against various threats including physical, chemical and biological stresses, such as wounding, exposures to salinity, drought, cold, heavy metals, air pollutants and ultraviolet rays and pathogen attacks, like fungi, bacteria and viruses. Plant reactions to these factors are very complex, and involve the activation of set of genes, encoding different proteins. These stresses can induce biochemical and physiological changes in plants, such as physical strengthening of the cell wall through lignification, suberization, and callose deposition; by producing phenolic compounds, phyto alexins and pathogenesis-related (PR) proteins which subsequently prevent various pathogen invasion. Among these, production and accumulation of pathogenesis related proteins in plants in response to invading pathogen and/or stress situation is very important. Phyto alexins are mainly produced by healthy cells adjacent to localized damaged and necrotic cells, but PR proteins accumulate locally in the infected and surrounding tissues, and also in remote uninfected tissues. Production of PR proteins in the uninfected parts of plants can prevent the affected plants from further infection. PR protein in the plants

was first discovered and reported in tobacco plants infected by tobacco mosaic virus. Later, these proteins were found in many plants. Most PR proteins in the plant species are acid-soluble, low molecular weight, and protease-resistant proteins. PR proteins depending on their iso electric points may be acidic or basic proteins but they have similar functions. Most acidic PR proteins are located in the intercellular spaces, whereas, basic PR proteins are predominantly located in the vacuole. The PR proteins have been classically divided initially into 5 families based on molecular mass, isoelectric point, and localization and biological activity. Currently PR-proteins were categorized into 17 families according to their properties and functions including β-1,3-glucanases, chitinases, thaumatin-like proteins, peroxidases, ribosome-inactivating proteins, defenses, thionins, nonspecific lipid transfer proteins, oxalate oxidase, and oxalate-oxidase-like proteins. Among these PR proteins chitinases and β-1, 3-glucanases are two important hydrolytic enzymes that are abundant in many plant species after infection by different type of pathogens. The amount of them significantly increase and play main role of defense reaction against fungal pathogen by degrading cell wall, because chitin and β-1,3-glucan is also a major structural component of the cell walls of many pathogenic fungi. β-1, 3 glucanases appear to be coordinately expressed along with chitinases after fungal infection.

Wheat *Mds-1* encodes a heat-shock protein and governs susceptibility towards the Hessian fly gall midge

Gall midges induce formation of host nutritive cells and alter plant metabolism to utilize host resources, that the gene Mayetiola destructor susceptibility-1 on wheat chromosome 3AS encodes a small heat - shock protein and is a major susceptibility gene for infestation of wheat by the gall midge M. destructor, commonly known as the Hessian fly. Transcription of Mayetioladestructor susceptibility-1 and its homoeologs increases upon insect infestation. Ectopic expression of Mayetiola destructor susceptibility-1 or induction by heat shock suppresses resistance of wheat mediated by the resistance gene *H13* to Hessian fly. Silencing of Mds-1 by RNA interference confers immunity to all Hessian fly biotypes on normally susceptible wheat genotypes.

Hessian fly infection induces Mds-1

On the basis of the EST CD453475 sequence, a full-length cDNA and the gene (GenBank Accession Code JN162442) were cloned from the susceptible wheat Newton by RACE-PCR and PCR. Mds-1 encodes a protein of 151 amino-acid residues and has 96% identity with a previously characterized HSP, HSP16.9, a member of a group of proteins with the α-crystallin domain. Without infestation, very low levels of Mds-1 transcript were detected in wheat leaf-sheaths, the feeding site for Hessian fly larvae. Higher transcript levels were found in developing grains. Both the transcript and protein levels increased in plants during compatible interactions following Hessian fly infestation, but no apparent increase in the transcript or protein levels was observed in plants during incompatible interaction with the wheat cultivar Molly, which carries the R gene *H13*.

Changes in properties of wheat leaf cuticle during interactions with Hessian fly

Infestation of wheat by Hessian fly larvae causes a variety of physical and biochemical modifications of the host plant. Changes occur in cuticle permeability, lipid composition and gene transcript abundance, and these responses differ substantially between resistant and susceptible wheat lines. Staining assays revealed that susceptible plants exhibited a generalized increase in leaf sheath epidermal permeability during infestation; whereas, epidermal permeability was only minimally affected in resistant plants. Furthermore, temporal profiling using gas chromatographic methods revealed that changes in cuticle lipid (wax and cutin) composition correlated well with differing levels of epidermal permeability in susceptible and resistant plants. Temporal analysis of cuticle-associated gene mRNA levels, by quantitative real-time PCR, indicated a relationship between transcript abundance and changes in cuticle lipid profiles of resistant and susceptible plants. Results suggest that conserving cuticle integrity via induction of specific wax constituents and maintenance of cutin amounts, determined by the accumulation of cuticle-associated transcripts, could be important components of wheat resistance to Hessian fly larvae.

A model for Mds-1 involvement in plant susceptibility

Hessian fly induces cells in the wheat sheath to develop into nutritive cells for the nourishment of fly larvae, which involves the conversion of host sheath cells to a nutritional sink. The process also induces a variety of stress-related genes, which initially were construed to reflect the host response to infection. However, the evidence presented here indicates that the Hessian fly specifically exploits the HSP gene Mds-1 and other related genes for the benefit of larval growth. Our results adds another dimension to the remarkable and ancient small HSP family proteins, including MDS-1, which are involved in a wide range of functions from construction of the animal eye lens to stress responses. The proliferation of small *HSP* genes in plants has been postulated to be an adaptation to dynamic environmental changes, including heat stress. Our results indicate that the Hessian fly and *B. graminis* f. sp. *tritici* exploit a heat shock-like response that leads to host susceptibility.

Remarkably, the Mds-1-silenced plants were observed to be poor hosts for the powdery mildew fungus B. graminis f. sp. tritici. Measurements of Mds-1 expression during infection of normal wheat plants revealed that Mds-1 is also induced to higher levels of expression during B. Graminis infection. The wheat variety Duster is resistant to many strains of *B. graminis* f. sp. *tritici*, including the KS-5 isolate, and very low Mds-1 expression was detected in Duster plants challenged by the fungus. Ectopic expression studies of Mds-1 were not conducted in the Duster variety. Nonetheless, heat stress of Duster led to both elevated levels of Mds-1 and the loss of resistance. The possibility exists that B. graminis f. sp. tritici specifically exploits the heat-shock pathway to suppress host immunity responses. The effect of Mds-1 silencing on susceptibility, however, did not extend to the leaf rust pathogen *Puccinia triticina* as silenced plants were equally susceptible to rust infection as normal plants.

The possible negative impact of Mds-1 silencing on wheat remains to be determined. Initial examination of apparent phenotypic abnormalities in Mds-1-silenced wheat lines include partial sterility, smaller grain weight, reduced plant height and low seed germination rates. However, abnormalities in plants with Mds-1 silenced vary from plant to plant and may have been caused by positional effects due to different integration sites of the Mds-1 construct into the wheat genome or by tissue culture. For practical application,

potential negative impact needs to be eliminated or reduced to minimum for economic benefit. One way to reduce potential negative impact is to use tissue-specific promoters for gene silencing. The rice S gene, Pi21, which encodes a transporter-like protein and is highly conserved among monocots, has been engineered for broad resistance to rice blast. Unlike R genes that have similar structures and possibly similar action modes, S genes exhibit greater variations in structures and functional mechanisms. The variation in S genes provides us opportunities for fundamental research to reveal mechanisms of plant susceptibility and resistance, as well as for practical applications to develop plants with improved resistance for pest management.

The effect of Mds-1 expression in wheat on resistance to Hessian fly and the loss of resistance to both Hessian fly and powdery mildew upon heat stress also provided insight into the resistance mechanisms. Numerous plant species with single major R genes lose resistance to herbivores under heat stress, suggesting a possible role of *Hsp* genes in plant susceptibility. The observations that a bacterial pathogen injects an HSP-like protein into host cells for virulence, and elevation in HSP70 levels induced by heat stress makes plants susceptible to an otherwise avirulent pathogen also support a role of HSPs in plant susceptibility. However, various HSPs including HSP90, HSP70, an HSP-like protein, and a small HSP have been found to interact with disease resistance protein complexes and are required for disease resistance. The basis for the role of MDS-1 and possibly other HSPs as well in plant susceptibility and the role of HSPs in plant resistance remains to be revealed. In a similar situation, receptor-like kinase genes are required for both fungal susceptibility and resistance in different plant–pathogen systems. The dominant effect of Mds-1 and possibly other temperature-dependent susceptibility genes under elevated temperatures pose a threat to the effectiveness of plant resistance to Hessian fly and other pests under scenarios of global climate change. A better understanding of the molecular mechanisms of Mds-1 and other temperature-dependent, dominant susceptibility genes is needed to preserve plant resistance in the face of global warming.

Reactive Oxygen Species Are Involved in Plant Defense against a Gall Midge

Reactive oxygen species (ROS) play a central role in plant defense against various pathogens, Superoxide anion (O2−), hydrogen peroxide (H2O2), and hydroxyl radical are the three major forms of ROS. These molecules are highly reactive and toxic and can lead to the oxidative destruction of cells. The rapid accumulation of plant ROS at the pathogen attack site, a phenomenon called oxidative burst, is toxic to pathogens directly and can lead to a hypersensitive response (HR) that results in a zone of host cell death, which prevents further spread of bio trophic pathogens. In addition to the described direct effects, ROS can also serve as signals that lead to the activation of other defense mechanisms. Several mechanisms have been proposed for ROS generation in plants. Of these mechanisms, the NADPH-dependent oxidase system has received the most attention because of its similarity to the mammalian oxidase system that initiates ROS production in phagocytes and B lymphocytes as a response to pathogen attack. In mammalian cells, NADPH-dependent oxidases catalyze the one-electron reduction of molecular oxygen to form O2−, which then undergoes dismutation to form H2O2 either spontaneously or catalyzed by superoxide dismutases. In plants, NADPH-dependent oxidases are also linked with O2− production in response to pathogen attack and wounding. Insertion mutations of two Arabidopsis (Arabidopsis thaliana) NADPH- dependent oxidase subunit genes, AtrbohD and AtrbohF, largely eliminate ROS production during disease resistance reactions to avirulent pathogens but evidence for their role in defense against insects is still preliminary and inconsistent. In this study, we examined the potential role of ROS in defense of wheat (*Triticum aestivum*) and rice (*Oryza sativa*) against Hessian fly (*Mayetiola destructor*) larvae. Rapid and prolonged accumulation of hydrogen peroxide (H2O2) was detected in wheat plants at the attack site during incompatible interactions. Increased accumulation of both H2O2 and superoxide was detected in rice plants during non host interactions with the larvae. No increase in accumulation of either H2O2 or superoxide was observed in wheat plants during compatible interactions. A global analysis revealed changes in the abundances of 250 wheat transcripts and 320 rice transcripts encoding proteins potentially involved in ROS homeostasis. A large number of transcripts encoded class III peroxidases that increased in abundance during both incompatible and non host interactions, whereas the levels of these transcripts decreased in susceptible wheat during compatible interactions. The higher levels of class III peroxidase transcripts were associated with elevated enzymatic activity of peroxidases at the attack site in plants during incompatible and non host interactions. Overall, our data indicate that class III peroxidases may play a role in ROS generation in resistant wheat and non host rice plants during response to Hessian fly attacks

Symptom Development

Phyto hormone Dynamics Associated with Gall Insects, and their Potential Role in the Evolution of the Gall-Inducing Habit

Plant galls can be induced by a variety of organisms, insects produce the most diverse and complex galls found in nature; yet, how these galls are formed is unknown. Phyto hormones have long been hypothesized to play a key role in gall production, but their exact role, and how they influence galls, has been unclear. Research in the past decade has provided better insight into the role of plant hormones in gall growth and plant defenses. We review and synthesize recent literature on auxin, cytokinins, and abscisic, jasmonic, and salicylic acids to provide a broader understanding of how these phyto hormones might effect gall production, help plants defend against galls, and/or allow insects to overcome host- plant defenses. After reviewing these topics, we consider the potential for phyto hormones to have facilitated the evolution of insect galls. More specialized research is needed to provide a mechanistic understanding of how phyto hormones operate in gall-insect-plant interactions, but current evidence strongly supports phyto hormones ask factors determining the success and failure of insect galls.

Phytohormones Involved in Defensive Responses Against Gall-inducing Insects

Our understanding of therole of phyto hormones in mediating plant defensive responses against insect herbivores has greatly improved in recent years. Most of our knowledge on this subject, however, comes from studies with ectophytic herbivores, like leaf-feeding caterpillars oraphids, and to date only a limited number of studies have focused on defenses against endophytic herbivores, and gall-inducing insects in particular. Phytohormones recognized to play important roles in mediating anti-herbivore defenses typically include

jasmonic acid (JA), salicylic acid (SA), ethylene and, as mentioned above, ABA, however, recent research indicates that hormones with other primary functions (e.g., auxins, giberellins, cytokinins, brassinosteroids, and peptide hormones) also can influence plant defensive responses against some pathogens and occasionally insect herbivores. The activities of plant hormones are fairly complex and various hormone combinations may have synergistic or antagonistic interactions; their combination, or hormone balance, determines the physiological outcomes. Insect feeding damage, especially from chewing herbivores, tends to trigger the jasmonate pathway, which regulates many downstream induced defensive responses, such as production of defense enzymes, synthesis of anti feedant chemicals, and synthesis and release of volatile compounds that can attract foraging natural enemies or deter additional oviposition by herbivores. Phloem-feeding herbivores that cause less severe tissue damage, on the other hand, are often perceived by plants similarly to pathogens, and they tend to activate the salicylic acid pathway, although JA and ethylene also may be induced. Gall-inducing insects include both chewing herbivores and those with piercing/sucking mouth parts, and there is evidence that JA-, SA-, and ABA-mediated plant defenses are involved against these herbivores. One challenge for studying plant defenses against gall insects is the degree of control or manipulation that gall inducers can exert over their host plants; thus, it is important to recognize that phyto hormone phenotypes may be altered by the gall insect, making it difficult to characterize a "normal" plant defensive response against a gall-inducing species. Valuable insight into the range of possible plant defensive responses can be gained via comparative frameworks that contrast the responses of one plant species to different herbivore species (including gallers), or responses of susceptible and resistant plant varieties to particular herbivore species. These comparisons can be profitable approaches for gaining insight into apparent phyto hormone manipulations that may influence gall formation or plant nutritional content.

Interactions with Defensive Hormones May Influence Gall-promoting Phyto hormones

As mentioned above, IAA and cytokinins appear to play a key role in gall initiation and formation, but it appears that gall insects also may gain other benefits from higher levels of IAA or cytokinins. Auxins are important regulators of many plant functions, such as vascular tissue differentiation, assimilate partitioning, and plant cell enlargement and division, but IAA also can interact with JA, and the two can inhibit each other's influence. Similarly, the influence of cytokinins, which promote cell division among other functions can also be inhibited by JA. By increasing IAA (or cytokinin) levels to induce gall formation, gall insects may be benefiting from lower levels of JA-mediated induced defenses. The gall-inducing caterpillar G. Gall aeso lidagin is induced high levels of IAA in its host plant *S. altissima*, but did not induce higher levels of the defensive hormones JA, SA, or ABA, perhaps because they were suppressed by IAA.

Similarly, feeding by the tephritid fly *E. solidaginis*, an insect known to foster high levels of IAA and cytokinins in its galls (Mapes and Davies 2001a, 2001b), also failed to induce higher levels of JA or SA or their associated downstream defenses (Tooker *et al*. 2008). Additionally, Hessian fly larvae feeding on wheat induced higher levels of IAA without increasing JA or JA-mediated defences (Tooker and De Moraes 2007, 2011b). Infact, in this wheat system, there was a significant negative relationship between IAA and JA concentrations, suggesting that higher IAA content may have negatively influenced the amount of JA in plant tissues, a finding that may provide insight into the evolution of the gall-inducing habit (Tooker and De Moraes 2011b). Further, a similar study exploring levels of phytohormones in wheat and rice (*Oryza sativa* L.) following Hessian fly attack detected a significant negative relationship between levels of IAA and 12-oxo-phytodienoic acid (OPDA; Zhu *et al*. 2011), which is a pre-cursor to JA and can induce plant defense responses on its own (Farmer and Ryan 1992). These results provide further evidence that IAA may negatively influence plant defenses, possibly by down-regulating the octadecanoid pathway, which generates JA. Because plant defenses exert such a strong selection pressure on insect herbivores (Ehrlich and Raven 1964), it is conceivable that gall insects initially evolved an ability to manipulate IAA and/or cytokinin levels, thus countering plant - defense responses, and the resulting hypertrophy could have been a secondary benefit (Tooker and De Moraes 2008, 2011b). As gall insects became more intimately associated with their host plants, and dependent on the specialized tissue of the gall, they likely were under equally strong pressure to avoid inducing levels of JA, which could inhibit plant growth and hypertrophy, thereby inhibiting gall formation (Tooker and De Moraes 2008). One could speculate that induction of JA, which is a powerful growth regulator (Meyer *et al*. 1984), would be an effective defensive response against gall insects 1) by inhibiting IAA and cytokinins and the cell growth and division associated with gall formation, and 2) through JAmediated, downstream anti-herbivore defenses (Fig. 1). Another line of evidence for the importance of JA-mediated defenses against gall insects comes from a study in which exposure to the pheromone of a gall-inducing fly primed JA induction in its host plant S. altissima (Helms *et al*. 2013). Similar to JA, IAA also is known to inhibit SA-mediated defenses. Thus, induction of higher levels of IAA may also effectively reduce SA defenses for some gall insects (Fig. 1). In fact, some plant pathogens manipulate auxin levels and overcome plant anti-pathogen defenses (Davies 2004; Kazan and Manners 2009; Navarro *et al*. 2006; Wang *et al*. 2007). These hormone interactions are likely to be complex, however, because negative cross-talk also exists between the JA and SA pathways.

Potential Role of Phyto hormones in Evolution of Gall Induction

One of the commonly asked questions about gall insects is "how did they evolve?" Most evidence indicates that the gall inducing habit evolved from other forms of endophytic feeding, such as leaf mining, stem boring, leaf rolling, or even parasitoids of these types of insects (Price 1992; Ronquist 1999; Roskam 1992). Other relevant questions that are critical to understanding evolution of gall insects are "how did gall insects develop the ability to induce production of their galls?" and "which traits predisposed predecessors of gall inducers to gain control of host- plant physiology?" While these questions remain to be fully answered, a few recent studies provide some insight into potential answers. First, as discussed above, atleast one leaf- mining caterpillar species possesses symbiotic bacteria that produce cytokinins that alter, or manipulate, host plant physiology to the benefit of the insect and the symbiont (Giron *et al*. 2007; Kaiser *et al*. 2010). Further, evidence from gall-inducing nematodes suggests that they may have acquired their galling abilities via horizontal gene transfer from bacteria or even soil- dwelling

fungi (Spíchal 2012); thus,even if symbionts are not directly responsible for phytohormone production of some gall-insect species, their acquired genes still may play a role. With this precedent established, it is tempting to believe that other gall-inducing species also may have gained an ability to produce phytohormones and manipulate their host plant via symbionts. It seems likely, however, that different lineages of insects have converged on different mechanisms of gall induction, and research will have to explore if other gall insects partner with symbionts or even use "borrowed" genes to force production of their galls. Second, given that gall-inducing sawfly larvae can synthesize IAA de novo (Yamaguchietal 2012), it may be that some gall insects can produce phytohormones on their own, there by manipulating their host plant to produce their gall. Endosymbionts may still contribute to IAA production, but details need to be clarified (Yamaguchietal 2012).

Third, there is evidence that endophytic species that do not form mines or induce galls also are able to alter host-plant physiology to their own benefit. European corn borer (ECB) larvae, for example, excrete high quantities of IAA, which alters the nutritional quality of stems and enhances larval growth (Dafoe *et al*. 2013). While the source of the IAA in larval frass remains to be identified (Dafoe *et al*. 2013), this finding with ECB is notable because, despite its relatively short evolutionary history with maize (~500yr; Bourguetetal. 2014), its feeding unexpectedly alters host-plant physiology and improves nutritional quality of its environment (Dafoe *et al*. 2013), a phenomenon not that different from what gall insects appear to do, suggesting that some degree of host-plant manipulation may be more common across plant-feeding taxa than currently realized. In fact, other taxa, likefree-living (i.e.not gall-inducing) aphids, also can alter the nutritional quality of their host plants (Sandström *et al*. 2000), and some insects even possess bacterial symbionts in their saliva that can suppress host plant anti-herbivore defences.

Because the ability to manipulate host plants appears somewhat distributed within phytophagous insect taxa, it is reasonable to hypothesize that the gall-inducing habit arose within lineages that had some ability to manipulate their host plants, likely via phytohormones. A generalized composite scenario that would be consistent with this hypothesis can be derived from existing literature. We propose that through random mutation or symbiosis, insect species developed an ability to manipulate or produce IAA or cytokinins, and these compounds improved the nutritional quality of their host plants (Kaiser *et al*. 2010; Tooker and De Moraes 2009, 2011b; Yamaguchi *et al*. 2012). Because these compounds also can negatively influence host-plant defenses (Saniewski *et al*. 2002; Ueda and Kato 1982), insects producing them may have gained a fitness advantage from both improved food quality and lower host-plant defences, thus establishing an evolutionarily stable state where the insects were successful (Tooker and De Moraes 2008, 2011). This evolutionary position then could have been exposed to biotic (natural enemies) or abiotic (desiccation) pressures that selected for covering galls of differing complexities (Stone and Schönrogge 2003), which further improved herbivore fitness. Finally, it should be recognized that there may be side effects of higher local concentrations of phytohormones, and these effects remain to be established as adaptive for the insect or not.

We acknowledge that this view of a potential path for evolution of gall induction may not be universal, will be challenging to test, and does not elucidate the exact mechanisms responsible for hypertrophy and hyperplasy other than implicating "phytohormones," but we feel it is a useful exercise to imagine potential evolutionary steps that could have developed features of the systems we witness today. Moreover, the phytohormones may be redirected from the plant, produced by symbionts, or the insects themselves; we have limited insight into how they alter patterns of cell growth and whether they interact with DNA directly or induce signaling cascades involving various genes and enzymes– many of these details must be elucidated by molecular biologists that choose to venture into the model systems developed to understand evolutionary ecology of gall insects (e.g., wheat/Hessian fly, Solidago /Eurosta or Gnorimoschema, Salix/Pontania, etc.). We also acknowledge that this conceptual model greatly simplifies the potential story, particularly from the perspective of the plant, which in our model may seem to be evolutionarily static and incapable of evolutionary change. In reality, this is, of course, not true as plants have very strong defensive capabilities and have co-evolved with their insect herbivores (Ehrlich and Raven 1964). Our goal here, however, was to consider evolution and phytohormone dynamics from the insect's perspective because gall insects have been broadly successful (i.e., evolved separately in many taxa), and articulating this hypothesis is an important step to laying the foundation for future research on the subject.

Role of mi RNAs and si RNAs in biotic and abiotic stress responses of plants:

Small, non-coding RNAs are a distinct class of regulatory RNAs in plants and animals that control a variety of biological processes. In plants, several classes of small RNAs with specific sizes and dedicated functions have evolved through a series of pathways. The major classes of small RNAs include microRNAs (miRNAs) and small interfering RNAs (siRNAs), which differ in their biogenesis. miRNAs control the expression of cognate target genes by binding to reverse complementary sequences, resulting in cleavage or translational inhibition of the target RNAs. siRNAs have a similar structure, function, and biogenesis as miRNAs but are derived from long double-stranded RNAs and can often direct DNA methylation at target sequences. Besides their roles in growth and development and maintenance of genome integrity, small RNAs are also important components in plant stress responses. One way in which plants respond to environmental stress is by modifying their gene expression through the activity of small RNAs. Thus, understanding how small RNAs regulate gene expression will enable researchers to explore the role of small RNAs in biotic and abiotic stress responses. This review focuses on the regulatory roles of plant small RNAs in the adaptive response to stresses.

Genes Expressed Differentially in Hessian Fly Larvae Feeding in Resistant and Susceptible Plants

The Hessian fly, *Mayetiola destructor*, is a destructive pest of wheat worldwide and mainly controlled by deploying resistant cultivars. The genes that were expressed differentially between larvae in resistant plants and those in susceptible plants through RNA sequencing on the Illumina platform. Informative genes were 11,832, 14,861, 15,708, and 15,071 for the comparisons between larvae in resistant versus susceptible plants for 0.5, 1, 3, and 5 days, respectively

Tissue and Life Stage Specificity of Glutathione S -

Transferase Expression in the HF, *Mayetiola destructor*: Implications for Resistance to Host Allelo chemicals:

1.1. Tissue-specific expression patterns of the *M. destructor* GSTs

Quantitative analysis of the *M. destructor* GST transcripts in larval tissues including midgut, salivary glands and fat body suggested their mRNA abundance to be tissue-specific in expression. The greatest levels of mRNA for *MdesGST-1* were observed in the fat body and midgut, whereas the mRNA levels for *MdesGST-2*and *MdesGST-3* were predominant in the midgut. The least level of transcriptional expression for all the *M. destructor* GSTs was found in the salivary gland samples and thus the expression in midgut and fat body were compared relative to the salivary glands. A significant ($p< 0.05$) fold difference of 2.6 and 2.3, respectively, was calculated for *MdesGST-1* in the fat body and midgut samples relative to the salivary gland tissue. Further, a fold change of 2.1 for *MdesGST-2* and 2.2 for *MdesGST-3* was calculated between the midgut and salivary gland tissues.

1.2. Developmental expression patterns of the *M. destructor* GSTs

Transcription profiling for the *M. destructor* GST genes was also performed for all the stages of development including the three larval instars, pupae and adults. Of all three *M. destructor* GSTs, mRNA for *MdesGST-1*was observed to be the most abundant, while mRNA for *MdesGST-3* was the least abundant. *MdesGST-1*showed an ascending pattern in mRNA levels during the larval instars, with a peak in the third instars. Interestingly, the expression profile for the Sigma GST (*MdesGST-2*) revealed a peak mRNA level in pupae. The lowest level of expression for all three *M. destructor* GSTs was observed in the first instar samples. Therefore, the fold change in mRNA abundance in the other developmental stages was calculated relative to this basal level in the first instars. Significant ($p<0.05$) fold differences of 3.2, 4.8, 1.8 and 1.9 for *MdesGST-1* were determined between second instar, third instar, pupa, and adult respectively compared to the first instar, while a 1.7-fold difference ($p<0.05$) for *MdesGST-2* was calculated between pupa and first instar. Levels of mRNA for *MdesGST-3* did not significantly vary throughout development.

Differential accumulation of phytohormones in wheat seedlings attacked by avirulent and virulent Hessian fly (Diptera: Cecidomyiidae) larvae

Many scientists analyzed the accumulation of six phytohormones and phytohormone-related compounds in a wheat, *Triticum aestivium* L., genotype, 'Molly', after attacks by avirulent and virulent Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), larvae, respectively, and we examined the expression of genes in the jasmonic acid (JA) pathway by Northern blot analysis. Compared with un infested plants, attacks by avirulent larvae resulted in increased accumulation of salicylic acid (SA) by 11.3- and 8.2-fold, 12-oxo-phytodienoic acid (OPDA) by 36.4-and 18.7-fold, 18:3 fatty acid by 4.5- and 2.2-fold, and 18:1 fatty acid by 1.8- and 1.9-fold at 24 and 72 h post-initial attack (hpia), respectively, but an 20% decrease in JA accumulation at 24 hpia at the attack site. Attacks by the virulent larvae did not affect the accumulation of SA, OPDA, and 18:3 and 18:1 fatty acids but dramatically increased the concentration of auxin (AUX) from undetectable in uninfested plants to 381.7 ng/g fresh weight at 24 hpia and 71.0 ng/g fresh weight at 72 hpia in infested plants. Transcript levels of genes encoding lipoxygenase 2, allene oxide synthase, and Arabidopsis storage protein 2 were increased after avirulent larval attacks but decreased after virulent larval attacks. Our results suggest that OPDA and SA may act together in wheat resistance to the Hessian fly, whereas AUX may play a role in the susceptibility of wheat plants. The increased OPDA accumulation after avirulent larval attacks was at least partially regulated through gene transcription.

Involvement of polyamines in plant disease resistance has been extensively reviewed. Polyamine catabolism produces H2O2, which plays a role in plant defense by contributing to the hypersensitive response that acts against different biotic stressors like fungi, bacteria and viruses. Some examples of polyamines associated with plant defense include castor (*Ricinus communis*) against *Fusarium oxysporum f. sp. ricini*, *Arabidopsis* against *Pseudomonas syringae* and tobacco in response to inoculation with Tobacco Mosaic Virus (TMV). Monocots also respond with increased polyamine levels during defense against microbial pathogens. In an incompatible interaction between barley and powdery mildew (*Blumeria graminis f. sp. hordei*), levels of free and conjugated spermidine and putrescine as well as activity of ODC, ADC and SAMDC enzymes increased, three days after inoculation.

Despite documented changes of plant polyamine levels in response to various microbial pathogens, limited information is available on their involvement in plant-pest interactions. Increased abundance of polyamines during plant resistance has been reported for interactions between sweet pepper and leaf miner and during tolerance in *Nicotiana attenuata* attacked bymirid bug and triticale infested by aphids. One proposed function in plant defense is that phenolic polyamines block glutamatergic neuromuscular junctions resulting in paralysis of insect skeletal muscles. Other defense mechanisms associated with increased polyamine abundance include spider mite-induced plant volatiles that attract carnivorous natural enemies to lima bean and disrupted settling of bird cherry-oat aphids on triticale.

Hessian fly (*Mayetiola destructor*), a member of the gall midge family (Cecidomyiidae) is a destructive insect pest of wheat (*Triticum aestivum*) causing significant economic losses worldwide. This insect is an obligate parasite that must receive all of its nutrition from the host plant. Following egg hatch, the first-instar Hessian fly larvae crawl down the leaf blade to the base (crown) of the wheat plant and attempt to establish sustained feeding sites. Probing by the larvae results in either an incompatible (avirulent larvae, resistant plant) or a compatible (virulent larvae, susceptible plant) interaction.

Resistance of wheat to Hessian fly attack is achieved through the action of any of distinct resistance genes (*H1-H34* plus *Hdic*) identified so far. Gene-for-gene interaction is thought to occur when a larval salivary gene product is recognized by a wheat resistance gene product. The resulting incompatible interactions are characterized by expression of defense response genes, accumulation of feeding deterrent proteins, and changes in surface wax composition as well as host-cell permeability that aids in delivery of these substances and ultimately leads to larval death.

During compatible interactions, salivary effectors from virulent larvae suppress wheat defense responses leading to susceptibility, which allows the insect to complete its life cycle. Within three to four days of larval attack, the virulent larvae alter host metabolic pathways resulting in differentiation of a nutritive tissue at the feeding site, which is

believed to provide the larvae a diet rich in essential nutrients. These physiological changes are accompanied by a shift from carbon-containing compounds to elevated levels of nitrogen-containing compounds with corresponding changes in transcript levels of genes involved in glycolysis, the pentose phosphate pathway, and the tricarboxylic acid cycle. The carbon/nitrogen shift may provide better nutrition for insect development. In addition, a significant increase in levels of certain amino acids, including, proline, glycine, serine, tyrosine and glutamic acid, were observed in nutritive tissue. Proline, glycine, serine and tyrosine are 'conditionally essential' amino acids, meaning they become essential only when the organism faces periods of extreme stress where the physiological need exceeds the organism's ability to produce. Although methionine abundance does not increase in compatible interactions, it is an essential amino acid that cannot be synthesized de novo by an animal and must be supplied in its diet. The demand for amino acids expands beyond the essential set to the conditionally essential set in rapidly developing insect tissues. Therefore, these nutrients must be supplied exogenously through diet. Proline, glutamic acid and methionine enter the ornithine biosynthesis pathway, eventually leading to the production of polyamines.

The present study focuses on the polyamine biosynthesis pathways in both wheat and Hessian fly larvae during compatible (susceptible plant) and incompatible (resistant plant) interactions. We addressed two hypotheses. The first hypothesis was that wheat production of polyamines would increase as a component of its defense response against attack by Hessian fly larvae. This assumption was based on numerous reports of polyamine accumulation in response of resistant plants to biotic stresses. The second hypothesis was that the polyamine biosynthetic pathway would be highly up-regulated in virulent Hessian fly larvae to support the rapid growth processes driven by gene transcription and translation, as is the case in organisms ranging from mammals to bacteria. We report differences in polyamine levels as well as in the transcript abundance of key genes involved in biosynthesis of polyamines in susceptible and resistant wheat plants during response to feeding by Hessian fly larvae. In addition, polyamine levels and biosynthetic pathway were monitored in virulent Hessian fly larvae. The implications of increased polyamines as an additional source of nutrition leading to development of the virulent Hessian fly larvae

Gall midges induce formation of host nutritive cells and alter plant metabolism to utilize host resources, show that the gene Mayetiola destructor susceptibility-1 on wheat chromosome 3AS encodes a small heat- shock protein and is a major susceptibility gene for infestation of wheat by the gall midge *M. destructor*, commonly known as the Hessian fly.

Transcription of Mayetiola destructor susceptibility-1 and its homologs increases upon insect infestation. Ectopic expression of Mayetiola destructor susceptibility-1 or induction by heat shock suppresses resistance of wheat mediated by the resistance gene *H13* to Hessian fly. Silencing of Mayetiola destructor susceptibility-1 by RNA interference confers immunity to all Hessian fly biotypes on normally susceptible wheat genotypes. (Liu *et al*, 2013)

Infestation of wheat by Hessian fly larvae causes a variety of physical and biochemical

Summary Points

Modifications of the host plant. Changes occur in cuticle permeability, lipid composition and gene transcript abundance, and these responses differ substantially between resistant and susceptible wheat lines.

Conclusion

The HF shares many features with biotrophic or hemibiotropic plant pathogens. In fact, it is remarkable to know how many HF plant-parasitic mechanisms closely resemble those of nematodes, fungi, and oomycetes. These include the manner in which the HF feeds on its host, its ability to modulate gene expression and the presence and structure of hundreds of putative effector-encoding genes in its genome.

1. Hessian fly interactions with wheat share important features with many plant pathogen and nematode interactions with plants, including a hemibiotrophic lifestyle, a sessile feedingstage, anarrowhostrange, minutemouthparts, aneffector-basedmechanismof attack, and ETI in the plant.
2. High-resolution genetic mapping utilizing the sequenced HF genome andan FPC-based physical map of the HF polytene chromosomes has permitted insect Avr gene mapping and discovery.
3. Hundreds of putative HF effector proteins exist in the HF genome. These show unmistakable signs of diversifying selection.
4. Wheat responds to HF attack with a qualitative resistance that is conferred by major resistance (H) genes. More than 32 H genes have been identified. The cloned Hdic gene has NBS-LRR motifs.
5. The histology of HF resistance resembles plant resistance to fungi. It involves a localized hyper sensitive reaction, the release of an oxygen burst, the fortification of the cell wall, and an upregulation of toxin-encoding genes.
6. Coevolutionary interactions between the Hessian fly and grasses are not constrained by major fitness costs, there being no fitness cost for H-gene-mediated resistance and a relatively small fitness cost for Hessian fly adaptation to plant resistance.

Future Issues

1. The roles that the Avr-gene-encoded effectors and other putative effectors play in both compatible and incompatible interactions have not been characterized.Wherearethese proteins localized in plant cells, how are they transported, and what are their cellular targets in the compatible interaction? Do Avr-gene-encoded proteins interact directly orindirectlywithH-geneproducts? Are the abundance and diversity of effector proteins associated with functional redundancy?
2. The resistance response to HF feeding in wheat is still relatively poorly understood. What is the sequence of downs tream plant responses that prevent Hessian fly larvae from feeding and eventually cause death? Do all HF R genes in wheat use the same resistance mechanisms and pathways? Do HF R genes mediate plant resistance to organisms other than the HF
3. Knowledge regarding the molecular mechanisms associated with HF resistance in wheat is forthcoming. How can this information be translated into durable plant resistance?
4. Comparative genomics provides an opportunity to understand the evolution of effector proteins. What effector proteins and motifs are conserved among Mayetiola gall midge species? Which, if any, of the effectors are effective in cells of different grass species? What are the evolutionary relationships among effectors

in more distantly related gall midges? Did gall midges obtain their effectors via horizontal gene transfer? Pheromone traps can be combined with PCR-based diagnostics for virulence and avirulence.

References

1. Anderson KG, Harris M O. Does R generesistance to Hessianfly allow wheat seedlings to escape larval-induced growth deficits? J. Econ. Entomol., 2006; 99:1842-53.
2. Cartwright WB, Caldwell RM, Compton LE. Responses of resistant and susceptible wheats to Hessian fly attack. Agron. J., 1959; 51:529-31.
3. Chen MS, Liu S, Wang H, Cheng X, Bouhssini ME, Whitworth RJ. Genes Expressed Differentially in Hessian Fly Larvae Feeding in Resistant and Susceptible Plants, Int. J. Mol. Sci., 2016; 17:1324.
4. Dsouza MR, Ravishankar BE. Nutritional sink formation in galls of *Ficus glomerata* Roxb. (Moraceae) by the insect *Pauropsylla depressa* (Psyllidae, Hemiptera), Tropical Ecology, 2014; 55(1):129-136.
5. Dylan KK, Nemacheck JA, Matthew AJ, Christie EW. Changes in properties of wheat leaf cuticle during interactions with Hessian fly. The plant journal, 2010; 63(1):31-43.
6. Harris MO, Freeman TP, Moore JA, Anderson KG, Payne SA, Anderson KM, Rohfritsch O. H-Gene-mediated resistance to Hessian fly exhibits features of penetration resistance to fungi. Phyto pathol, 2010; 100:279-289.
7. Hatchett JH, Gallun RL. Genetics of the ability of the Hessian fly, Mayetiola destructor, to survive on wheats having different genes for resistance. Ann Entomol Soc Am., 1970; 63:1400-7.
8. Mcdonald MJ, Ohm HW, Rinehart KD, Giovanini MP, Williams CE. H33: A wheat gene providing Hessian fly resistance for the southeastern United States. Crop Sci., 2014; 54:1-9.
9. Omprakash M, Jonathan JN, Richard HS., Antioxidant defense response in a galling insect, PNAS. 2006; 6:1889–1894.
10. Omprakash M, Jonathan JN, Richard HS. Tissue And Life Stage Specificity of Glutathione S-Transferase Expression in the Hessian Fly, Mayetiola destructor: Implications for Resistance to Host Allelochemicals, J Insect sci., 2007; 7:20.
11. Shah J. Lipids, lipases, and lipid-modifying enzymes in plant disease resistance. Annu. Rev.
12. Phytopathol, 2005; 43:229-260.
13. Stuart M, Shun C, Richard S, Harris MO. Gall midges (Hessian flies) as plant pathogens. Annual review of phytopathology, 2012; 50:339-57
14. Subramanyam SS, Sardesai N, Minocha CS, Cheng Zheng, Richard HS, Christie EW. Hessian fly larva triggers polyamine levels in susceptible but not in resistant wheat. BMC Plant Biology, 2015; 15:3.
15. Xuming L, Chitvan K, Jiarui L, Harold NT, Huang L, Bikram SG, Gerald RR, Ginny A, Frank FW, Ming–Shun C *et al*. Rapid mobilization of membrane lipids in wheat leaf sheaths during incompatible interactions with Hessian fly, Nature Communications, 2013; 25(7):920-930