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Hessian fly: New insights to develop a gall nutritive tissue

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Abstract
Gall midges constitute an important group of insects.it is plant parasitic. The Hessian fly (HF; *Mayetiola destructor*), it is the gall midge, was the first insect to have a gene-for- gene interaction with its host plant i.e. wheat (*Triticum aestivum* spp.). The Hessian fly will produce nutritive tissue probably acts as a sink tissue within the wheat seedling, and it will not produce any galls, benefiting the growth of larvae by importing photo assimilates. Breakdown of nutritive cells began soon after they were first observed, indicated by a change in the shape and density of the cell nucleus, 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, virulent gene

Introduction
The HF is one of the most important gall midges. Its larvae live on grasses. Its biology resembles that of the majority of cecidomyiids, because of its attack of wheat seedlings, and upto 50 larvae can survive on the same seedling (gall), it is the most difficult one to propagate 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. Females will lay 200 eggs on upper 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 flax seed stage. HF adults enclose from the puparia and live for only one to four days.

Pest 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
Plant should survive in the nature, so it has to detect reactions which will take place in the plant by herbivore. Thus, after the 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 [4].

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 the and are restricted to the treated leaf tissue.

Moreover, the 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 have 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 flg 22, 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 [4].

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 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 [7] and alkaline phosphatase [8] 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 [11].

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. 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 the tissue formation largely remain unknown. It is thought that components of the larvae's saliva trigger change 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 [6].

The wheat–hessian Fly interaction

Interactions between plants and their parasitic fungi, oomycetes, and nematodes suggest that plant immunity has required each group of parasites to converge on a similar effector - based mechanism of attack [9].

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 penetrate into the cell wall. The tip of the mandible resembles the end of a hypodermic needle; it grooves 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 analogous to 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 [16].

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 the 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 [16].

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 with 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). 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

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, and an emerging model for all gall midges, results provide an opportunity to understand the cooperative antioxidant defense responses in the Hessian fly/ wheat interaction and may be applied to other insect/plant interactions [14].

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 wheat 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 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 were 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: Pathogenesis-related (PR) proteins are proteins produced in plants in the event of a pathogen attack. They are induced as part of systemic acquired resistance. Infections activate genes that produce PR proteins. Some of these proteins are antimicrobial, attacking molecules in the cell wall of a bacterium or fungus.

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 Mayetiola destructor 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 [19].

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 an incompatible interaction with the wheat cultivar Molly, which carries the R gene *H13* [18].

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.

A better understanding of the molecular mechanisms of Mds-1 and other temperature-dependent, dominant susceptibility genes are needed to preserve plant resistance in the face of global warming [19].

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. H2O2 or superoxide accumulation was not observed in wheat plants during compatible interactions.

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.

Similarly, feeding by the tephritid fly *E. solidaginis*, an insect known to foster high levels of IAA and cytokinins in its galls, also failed to induce higher levels of JA or SA or their associated downstream defenses. Additionally, Hessian fly larvae feeding on wheat induced higher levels of IAA without increasing JA or JA-mediated defences [17]. 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 [17]. 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 which is a pre-cursor to JA and can induce plant defense responses on its own [11]. 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 [19]. 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 [19].

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 (si RNAs), 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 stress.

Tissue and Life Stage Specificity of Glutathione S - Transferase Expression in the HF, *Mayetiola destructor* Implications for Resistance to Host Allelo chemicals

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.

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.

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

In the wheat plant there was an accumulation of phyto hormones and phyto hormone related compound accumulation after hessian fly attacks both by avirulent and virulent. 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 responds to 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 the 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 by mirid 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 35 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 the 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 the development of the virulent Hessian fly larvae. Gall midges induce formation of host nutritive cells and alter plant metabolism to utilize host resources, so that the gene *Mayetiola destructor* susceptibility-1 on wheat chromosome 3AS encodes hsp 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 the 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 [19].

Conclusion

The HF shares many features with bio trophic or hemibio tropic 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 genes

1. Hessian fly interactions with wheat share important features with many plant pathogen and nematode interactions with plants, including a hemibio trophic lifestyle, a sessile feeding stage, anarro who strange, minute mouth parts, an effector-based mechanism of attack, and ETI in the plant.
2. High-resolution genetic mapping utilizing the sequenced HF genomeandan FPC-based physical map of the HF polytene chromosomes has permitted insect Avr gene mapping and discovery
3. Hundreds of putative HF effect or proteins exist in the HF genome. Theses how 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 up regulation of toxin- encoding genes.
6. Co evolutionary 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. Where are these 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 orin directly with H-gene products? 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 down-stream plant responses that prevent He ssianfly 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?
5. Pheromone traps can be combined with PCR-based diagnostics for virulence and avirulence.

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