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e-Xtra*

Rapid Mobilization of Membrane Lipids in Wheat Leaf Sheaths During Incompatible Interactions with Hessian Fly

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Hessian fly (HF) is a biotrophic insect that interacts with wheat on a gene-for-gene basis. We profiled changes in membrane lipids in two isogenic wheat lines: a susceptible line and its backcrossed offspring containing the resistance gene *H13*. Our results revealed a 32 to 45% reduction in total concentrations of 129 lipid species in resistant plants during incompatible interactions within 24 h after HF attack. A smaller and delayed response was observed in susceptible plants during compatible interactions. Microarray and real-time polymerase chain reaction analyses of 168 lipid-metabolism-related transcripts revealed that the abundance of many of these transcripts increased rapidly in resistant plants after HF attack but did not change in susceptible plants. In association with the rapid mobilization of membrane lipids, the concentrations of some fatty acids and 12-oxo-phytodienoic acid (OPDA) increased specifically in resistant plants. Exogenous application of OPDA increased mortality of HF larvae significantly. Collectively, our data, along with previously published results, indicate that the lipids were mobilized through lipolysis, producing free fatty acids, which were likely further converted into oxylipins and other defense molecules. Our results suggest that rapid mobilization of membrane lipids constitutes an important step for wheat to defend against HF attack.

The wheat–Hessian fly (HF) (*Mayetiola destructor*) system has become a model for studies on plant–gall midge interactions (Stuart et al. 2008). Because of its importance in agriculture, the virulence and genetics of HF have been studied extensively (Harris et al. 2003; Stuart et al. 2008). HF attack induces reprogramming of gene transcription and dramatic changes in metabolic pathways in wheat (Liu et al. 2007; Zhu et al. 2008). In susceptible wheat during compatible interactions, nutritive cells are formed at the feeding site (Harris et al. 2006), and metabolic pathways are engineered toward producing more amino acids for HF nutrition (Zhu et al. 2008). Growth of susceptible plants is inhibited after HF infestation and the infested plants die eventually, after HF larvae no longer need nutrients from the host (Byers and Gallun 1971). On the other hand, HF larvae die in plants containing an effective resistance (*R*) gene, and the attacked plants continue to grow normally after some initial growth deficit (Anderson and Harris 2006). Substantial indirect evidence suggests that HF attacks wheat using salivary secretions (Byers and Gallun 1971; Chen et al. 2008, 2010; Hatchett et al. 1990), which are likely to target regulatory pathways in wheat to induce changes that benefit the insect, as observed with many secreted elicitors in pathogen–plant interactions (Bent and Mackey 2007; Hueck 1998).

Defense in wheat is activated on a typical gene-for-gene basis in response to HF attack (Hatchett and Gallun 1970), with resistance conditioned by major dominant *R* genes (Hatchett and Gallun 1970; Liu et al. 2005). Avirulence in HF is controlled by dominant avirulence (*Avr*) genes (Behura et al. 2004). Because the HF–wheat interaction is similar to gene-for-gene interactions observed in many pathogen–plant systems, it is likely that an *Avr* gene encodes a virulence factor, which is recognized either directly or indirectly by the corresponding R protein once it is secreted into host tissue during larval feeding (Bent and Mackey 2007; Keen 1990). The specific recognition between a pair of R and Avr proteins results in defense signaling and, eventually, the launch of direct chemical defenses, resulting in antibiosis to HF larvae (Chen et al. 2009b; Harris et al. 2010).

Antibiosis in resistant plants during incompatible interactions is likely due to a combination of mechanisms, including toxic chemicals such as lectins (Subramanyam et al. 2008), reactive oxygen species (Liu et al. 2010), digestive inhibitors (Wu et al. 2008), and secondary metabolites (Liu et al. 2007). Strengthening of the cell wall by increased deposits of phenolics (Liu et al. 2007) and enhanced cuticle wax (Kosma et al. 2010) are also likely involved in antibiosis. In previous studies, we found that several genes involved in lipid metabolism were differentially regulated in plants during incompatible and compatible interactions (Liu et al. 2007). Similar results were also observed in the wheat–Russian wheat aphid interaction (Smith et al. 2010). These observations suggest that lipids may play an important role in wheat defense against HF and other insect pests.

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Polar lipids are major components of membranes. Eukaryotic membranes not only form mechanical barriers to separate the interior of a cell or a subcellular structure from the outside environment but also serve as critical interfaces for interactions between cellular structures, cells, and organisms. Membranes consist of diverse lipids, and lipid composition changes in response to either internal signals or outside stimuli (McMahon and Gallop 2005). During interactions between plants and herbivores, lipid changes in cellular membranes of the attacked host could be critical for defense signaling (Laxalt and Munnik 2002) and the launch of direct defenses (Kosma et al. 2010). Until recently, however, analysis of compositional changes in membrane lipids has been labor intensive. Because of the technical difficulty, studies on the roles of lipids in plant defense against herbivores have been focused on analysis of individual lipid species or a subset of lipids (Saini et al. 1994; Seigler 1983). The development of electrospray ionization mass spectrometry (ESI-MS) has made possible the detection and quantification of membrane lipids (Forrester et al. 2004; Welti et al. 2002). The sensitivity, accuracy, and reproducibility of ESI-MS make it a powerful tool for systematically profiling changes in lipids in organisms under various conditions.

The objectives of this study were to i) profile lipid compositions in wheat seedlings of two different genotypes, a susceptible line and a backcrossed offspring containing the R gene *H13*; ii) investigate temporal changes in membrane lipids in resistant and susceptible wheat plants after HF attack; iii) analyze changes in abundance of transcripts encoding proteins involved in lipid metabolic pathways; and iv) examine whether downstream metabolites of membrane lipids play a role in wheat defense against HF. We provide compelling evidence that a significant portion of membrane lipids were mobilized for plant defense in resistant plants during incompatible interactions.

RESULTS

Lipid composition in leaf sheath of wheat seedlings.

'Newton' (the susceptible parent) and 'Molly' (the backcrossed offspring carrying the *R* gene *H13*) (Patterson et al. 1994) were grown under normal growth conditions and profiled for lipid content at 10 to 11 days after germination (Table 1), when HF infestation took place. In total, 129 lipid species were detected in the second leaf sheath (10 mm of tissue above the base of the first leaf node) of wheat seedlings. The detected lipids included 14 apparent molecular species (as defined by total acyl carbons/total carbon-carbon double bonds) of digalactosyldiacylglyerol (DGDG), 15 species of monogalactosyldiacylglycerol (MGDG), seven species of phosphatidylglycerol (PG), 20 species of phosphatidylcholine (PC), 20 species of phosphatidylethanolamine (PE), 10 species of phosphatidylinositol (PI), 20 species of phosphatidylserine (PS), nine species of phosphatidic acid (PA), five species of lysophosphatidylglycerol (lysoPG), five species of lysophosphatidylcholine (lysoPC), and four species of lysophosphatidylethanolamine (lysoPE). PC and PE were the two major groups, representing 41 to 52 and 22 to 25% of total detected lipids, respectively. DGDG, MGDG, PG, PI, PS, and PA were also relatively abundant, each group representing 1 to 8% of total detected lipids. The three groups of lysophospholipids—lysoPG, lysoPC, and lysoPE—were also detected but their concentrations were very low, each group representing less than 0.3% of total detected lipids. No statistically significant difference was observed in lipid concentrations between Newton and Molly and between day-10 and day-11 samples.

In terms of acyl chains, diacyl lipids with 32, 34, 36, 38, 40, and 42 total acyl carbons were detected (Supplementary Table S1). Lipids with 34 and 36 carbons were predominant, each group representing 42 to 53%, respectively. Lipids with 38 carbons were approximately 2% and lipids with all other acyl chain combinations together accounted for less than 1%. Again, there were no statistically significant differences in the concentrations of lipids with specific acyl groups between the two plant genotypes and between the two timepoints. A complete list of the 129 lipids and their concentrations in the second leaf sheath is given in Supplementary Table S2.

HF attack decreases the concentrations of total lipids and most lipid subgroups.

A preliminary analysis indicated that genes coding for lipases and lipid transfer proteins were upregulated in resistant plants within an hour following HF infestation (data not shown). Accordingly, a time course consisting of 0.5, 3, 6, 12, and 24 h was designed for the assay. The total concentration of detected lipids was reduced in resistant plants during incompatible interactions between 3 and 24 h after HF attack (Fig. 1). Specifically, an average of 32.7, 25.9, 44.6, and 38.9% reduction was observed in infested tissue at 3, 6, 12, and 24 h, respectively. No significant difference was observed between uninfested and

Table 1. Lipid classes detected in the second leaf-sheath of wheat seedlings at day 10 and day 11 after germination[a]

	Apparent lipid species[b]		Plants at 10 days after germination				Plants at 11 days after germination			
	Species		Newton		Molly		Newton		Molly	
Class[c]	Number	In class (%)	Conc.	Lipids (%)	Conc.	Lipids (%)	Conc.	Lipids (%)	Conc.	Lipids (%)
DGDG	14	10.9	5.7 ± 0.5	6.2	5.7 ± 0.6	6.1	4.7 ± 0.4	4.9	4.2 ± 0.2	5.1
MGDG	15	11.6	7.2 ± 0.9	7.8	7.1 ± 1.1	7.5	5.1 ± 1.2	5.3	4.3 ± 0.1	5.3
PG	7	5.4	4.8 ± 0.2	5.2	5.0 ± 0.3	5.3	5.0 ± 0.3	5.1	4.1 ± 0.5	5.0
PC	20	15.5	44.1 ± 4.8	47.6	43.7 ± 5.0	46.3	49.7 ± 3.8	51.5	41.3 ± 7.4	50.7
PE	20	15.5	21.7 ± 0.5	23.4	23.4 ± 1.0	24.8	22.1 ± 1.1	22.9	19.2 ± 1.5	23.5
PI	10	7.8	6.6 ± 0.3	7.1	7.0 ± 0.4	7.4	6.9 ± 0.7	7.2	5.4 ± 0.5	6.6
PS	20	15.5	1.1 ± 0.1	1.2	0.8 ± 0.1	0.9	1.2 ± 0.2	1.2	1.1 ± 0.2	1.4
PA	9	7	1.0 ± 0.3	1.1	1.3 ± 0.4	1.4	1.3 ± 0.3	1.3	1.6 ± 0.2	2.0
LysoPG	5	3.9	0.0 ± 0.0	0.0	0.01 ± 0.0	0.0	0.1 ± 0.1	0.1	0.0 ± 0.0	0.0
LysoPC	5	3.9	0.1 ± 0.0	0.2	0.2 ± 0.0	0.2	0.2 ± 0.0	0.2	0.2 ± 0.0	0.2
LysoPE	4	3.1	0.2 ± 0.0	0.2	0.2 ± 0.0	0.3	0.3 ± 0.0	0.3	0.2 ± 0.0	0.3
Total	129	100	92.6 ± 4.6	100	94.5 ± 4.5	100	96.5 ± 4.9	100	81.5 ± 9.3	100

[a] Conc. = concentration (nmol/mg of dry mass, average value of four biological replicates with standard error of the mean) and Lipids = percentage of total lipids.

[b] Apparent lipid molecular species per class.

[c] DGDG = digalactosyldiacylglyerol, MGDG = monogalactosyldiacylglycerol, PG = phosphatidylglycerol, PC = phosphatidylcholine, PE = phosphatidylethanolamine, PI = phosphatidylinositol, PS = phosphatidylserine, PA = phosphatidic acid, LysoPG = lysophosphatidylglycerol, LysoPC = lysophosphatidylcholine, and LysoPE = lysophosphatidylethanolamine.

infested tissues of resistant plants during incompatible interactions at 72 h after HF attack, indicating that the reduction in lipid concentration at earlier timepoints was restored. In comparison, total concentration of detected lipids did not change significantly in susceptible plants during compatible interactions at all timepoints after HF attack.

The impact of HF attack on different subgroups of lipids in resistant plants during incompatible interactions was relatively consistent, a significant reduction in concentrations of all subgroups at all timepoints, with three exceptions (Table 2). The three exceptions were lysoPG (not affected within the tested period), PA (not affected except for the 24 h timepoint), and lysoPC (increased slightly at 0.5 h timepoint). In comparison, the impact of HF attack on different lipid subgroups in susceptible plants during compatible interactions varied from subgroup to subgroup. Many lipid subgroups either did not change or increased in concentration in susceptible plants after HF attack. For those lipid subgroups that did show a decrease in concentration, the percentage of decrease was often much smaller than that in resistant plants.

HF attack affects the concentrations of individual lipid species differentially.

The distribution and severity of individual lipids affected by HF attack were quite different between resistant and susceptible plants (Supplementary Table S3; Supplementary Fig. S1). More lipids had reduced concentration in resistant plants in comparison with those in susceptible plants. In addition, the effect of HF attack on lipids was evident earlier and with greater magnitude in resistant plants than in susceptible plants. Specifically, there were 7, 31, 25, 52, and 46 individual lipids with concentration reduced in resistant plants at 0.5, 3, 6, 12, and 24 h after HF attack, respectively. Only four lipids across all timepoints (one at 0.5 h, two at 6 h, and one at 24 h) exhibited an increase in concentration in resistant plants after HF attack. On the other hand, there were 0, 0, 11, 11, and 31 lipid species with concentration reduced in susceptible plants at 0.5, 3, 6, 12, and 24 h after HF attack, respectively. Fourteen lipids (two at 0.5 h, five at 12 h, and seven at 24 h) exhibited an increase in concentration in susceptible plants. Most galactolipid species (DGDG and MGDG) were affected by HF attack in plants during both incompatible and compatible interactions. The number of phospholipids affected by HF attack varied, and only a few lipids in PG, PA, and PE subgroups were negatively affected.

HF attack upregulates lipid metabolism-related genes in resistant wheat.

To examine the impact of HF attack on the level of transcripts of lipid metabolism-related genes, primer pairs (Supplementary Table S4) for nine wheat transcripts were designed according to representative cDNA sequences, and quantitative real-time polymerase chain reaction (PCR) (qPCR) was carried out to determine the abundance of these transcripts. Three lipase-encoding transcripts were analyzed, including a GDSL-motif lipase (Fig. 2A), an extracellular lipase (Fig. 2B), and a triacylglyerol lipase gene (Fig. 2C). The abundance of all three transcripts increased greatly in resistant plants during incompatible interactions at some point after HF attack. The increase of the GDSL-motif lipase transcript was most dramatic, with over 1,000-fold at 0.5 h. On the other hand, the abundance of these three transcripts either decreased, remained the same, or slightly increased in susceptible plants during compatible interactions.

Three phospholipase gene transcripts were analyzed, including a phospholipase A2 (Fig. 2D), a phospholipase C (Fig. 2E), and a phospholipase D (Fig. 2F). HF attack affected the abundance of these transcripts in both resistant and susceptible plants. Specifically, the abundance of phospholipase A2 tran-

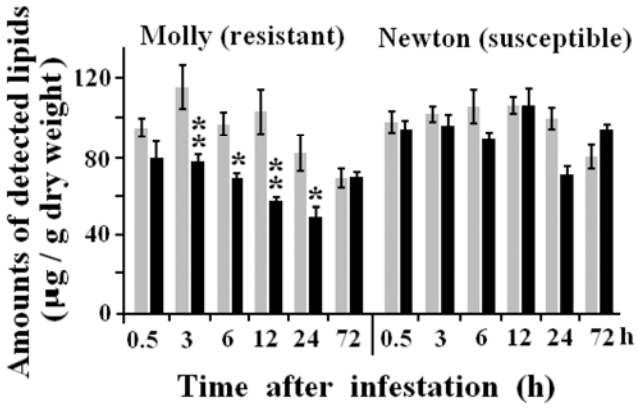

Fig. 1. Hessian fly (HF) attack reduces total detected membrane lipids in resistant plants during incompatible interactions. Samples were prepared from uninfested controls (gray bars) and HF-infested plants (black bars) at 0.5, 3, 6, 12, 24, and 72 h. Single and double asterisks (*) indicate differences between uninfested and infested samples statistically significant at *P* values of 0.05 and 0.01, respectively, on Tukey's honestly significant difference test. Standard errors of the means were shown in each bar.

Table 2. Impact of Hessian fly (HF) attack on lipid classes in resistant and susceptible plants at different timepoints after HF attack

	Percentage change in lipid concentration in plants attacked by HF larvae[a]									
	0.5 h		3 h		6 h		12 h		24 h	
Group[b]	R	S	R	S	R	S	R	S	R	S
DGDG	–23.97	–1.61	–40.3	–6.8	–35.25	–17.56	–49.7	–17.47	–47.44	–38.91
MGDG	–9.61	+4.54	–33.92	–11.94	–26.45	–18.2	–39.77	–22.47	–40.14	–33.69
PG	–17.7	–5.92	–32.2	–8.15	–23.15	–13.76	–40.31	–6.15	–34.05	–31.95
PC	–20.58	–11.45	–37.44	–15.79	–29.34	–21.77	–51.28	–11.66	–41.15	–37.5
PE	–8.76	+6.32	–22.53	+8.92	–16.3	+2.33	–29.84	+26.49	–29.22	–7.48
PI	–20.53	+3.6	–29.86	+5.36	–29.96	–6.05	–49.43	+15.96	–47.68	–17.75
PS	–33.41	–18.43	–42.67	–4.09	–44.54	–27.51	–63.31	–3.84	–58.92	–34.75
PA	NSC	NSC	NSC	NSC	NSC	NSC	NSC	+118.78	–42.31	+186.4
LysoPG	NSC	NSC	NSC	NSC	NSC	NSC	NSC	NSC	NSC	NSC
LysoPC	+0.56	+40.28	–7.25	+17.35	–8.79	+15.59	–23.74	+62.7	–31.28	+12.63
LysoPE	–16.18	+4.00	–27.46	+8.47	–21.52	+11.11	–34.96	+51.95	–33.64	+12.98

[a] NSC = no significant change ($P \geq 0.05$), R = resistant plants during incompatible interactions, and S = susceptible plants during compatible interactions. A positive percentage represents an increase whereas a negative percentage represents a decrease in lipid concentration following HF attack. Statistical analysis was conducted as described in Materials and Methods. The significance level is 0.05.

[b] Lipid groups. DGDG = digalactosyldiacylglyerol, MGDG = monogalactosyldiacylglycerol, PG = phosphatidylglycerol, PC = phosphatidylcholine, PE = phosphatidylethanolamine, PI = phosphatidylinositol, PS = phosphatidylserine, PA = phosphatidic acid, LysoPG = lysophosphatidylglycerol, LysoPC = lysophosphatidylcholine, and LysoPE = lysophosphatidylethanolamine.

script increased approximately sixfold in resistant plants at 0.5 h after HF attack, and increased approximately twofold thereafter. The abundance of this phospholipase A2 transcript also increased approximately twofold in susceptible plants throughout the experimental time period. The impact of HF attack on the phospholipase C transcript was similar to that on the phospholipase A2 transcript but to a more moderate degree. The abundance of the phospholipase D transcript increased slightly at the 0.5- and 6-h timepoints in resistant plants, and increased approximately onefold (i.e., twofold change) at the 6-h timepoint in susceptible plants.

Three transcripts, each encoding a different lipid transfer protein, were also analyzed (Fig. 2G to I). The abundance of transcript AF334185 (GenBank accession number) increased 1.5- to 8-fold in resistant plants but decreased 0.5- to 2-fold in susceptible plants (Fig. 2G). The abundance of transcripts CD896324 (Fig. 2H) and CN012788 (Fig. 2I) did not change in resistant plants but decreased two- to eightfold in susceptible plants.

HF attack upregulates lipid metabolism-related pathways coordinately in resistant wheat.

To gain insight on the molecular basis for lipid reduction in resistant plants during incompatible interactions and possible products to which the mobilized lipids were converted, changes in the transcript abundance of genes potentially involved in lipid metabolism were analyzed through microarrays (Table 3). In total, 168 probe sets were included in the analysis, including 43 from transcripts putatively involved in lipolysis (various lipases), 55 from transcripts likely involved in lipid transport (lipid trans-

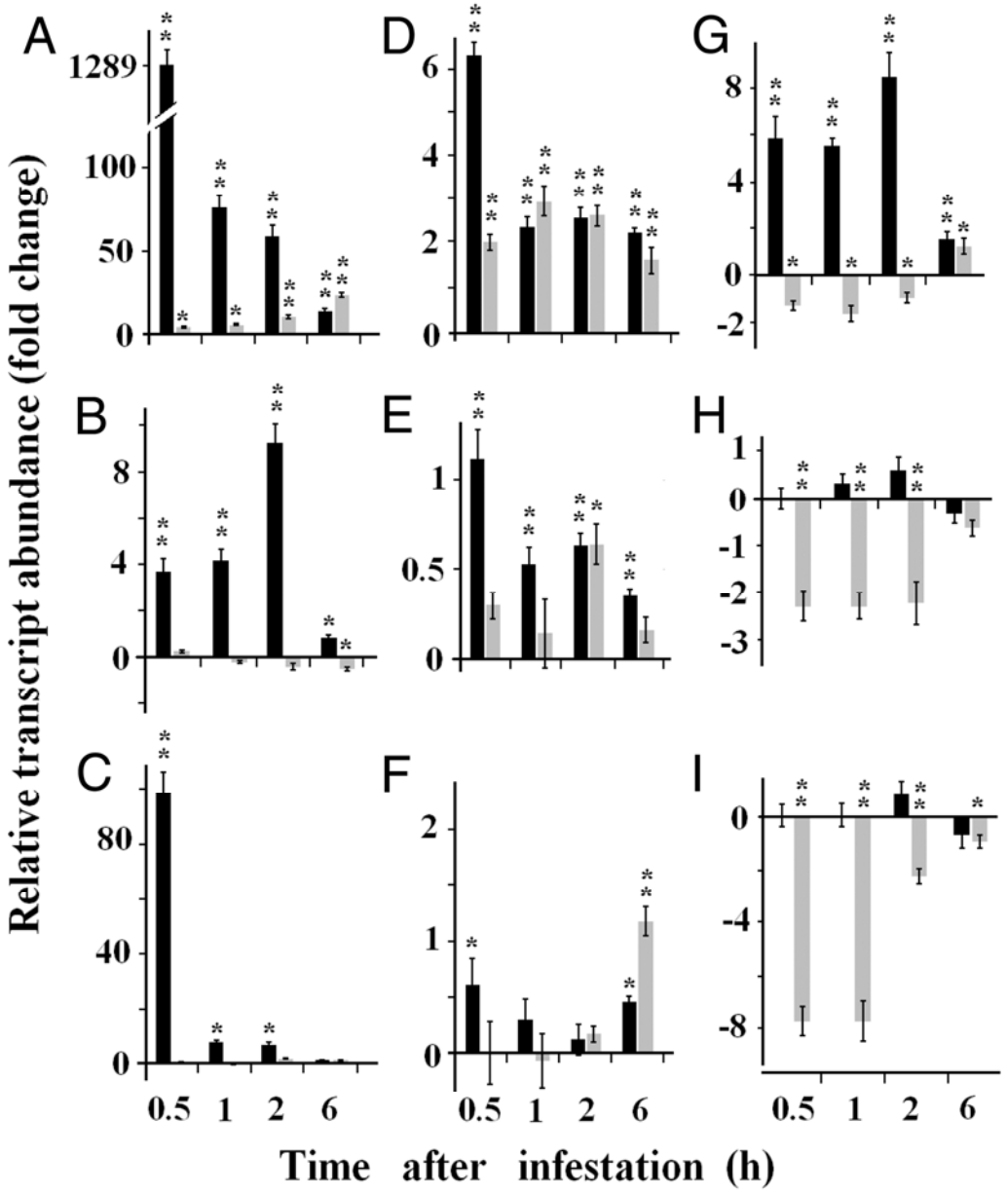

Fig. 2. Hessian fly (HF) attack upregulates transcript abundance of lipid metabolism-related genes. Quantitative polymerase chain reaction was carried out with samples prepared from 0.5, 1, 2, and 6 h after the initial HF larval attack. Solid bars represent fold changes in resistant plants. Gray bars represent fold changes in susceptible plants. Single and double asterisks (*) indicate $P \leq 0.05$ and 0.01, respectively. **A,** Gene encoding a putative GDSL-motif lipase (BJ246034). **B,** Gene encoding a family II extracellular lipase 1 (GenBank accession number BQ171153). **C,** Gene encoding a triacylglycerol lipase (BE493028). **D,** Gene encoding a phospholipase A2 (CV772474). **E,** Gene encoding a phosphatidyl glycerol phospholipase C (BQ171283). **F,** Gene encoding phospholipase D (AJ880008). **G,** Gene encoding a putative lipid transfer protein (AF334185). **H,** Gene encoding another lipid transfer protein (CD896324). **I,** Gene encoding a putative type 1 nonspecific lipid transfer protein (CN012788).

fer proteins), 31 from transcripts related to oxylipin synthesis, 27 from gene transcripts related to wax synthesis, five from gene transcripts encoding putative enzymes for fatty acid synthesis, and seven from gene transcripts encoding putative enzymes for fatty acid catabolism. Alteration in the abundance of individual transcripts after HF attack is shown in Supplementary Table S5. Changes in different groups of transcripts at different timepoints are summarized in Table 3.

Among 43 lipase transcripts, 22 increased in abundance in resistant plants at some point during the 3-day period after HF attack, whereas only five transcripts decreased. In contrast, only one transcript increased and as many as nine transcripts decreased in abundance in susceptible plants at some point during the same period. Transcript abundance was affected the most during the first 24 h. Among the different types of lipases, transcripts for lipase class 3 (triacylglycerol lipase) and GDSL-motif lipases were affected the most. Only a small number of phospholipase transcripts were affected.

Among the 55 transcripts encoding putative lipid transfer proteins, 27 of them increased and nine decreased in abundance in resistant plants after HF attack. In contrast, none of the transcripts increased and 29 decreased in susceptible plants after HF attack (Table 3). Most of the transcripts that increased in abundance in resistant plants showed a decrease in susceptible plants. The opposite changes resulted in dramatic differences in the abundance of these transcripts between resistant and susceptible plants.

Among the 31 transcripts potentially involved in oxylipin synthesis, 23 increased and 8 decreased in abundance in resistant plants after HF attack (Table 3). One of them increased more than 600-fold. In contrast, no oxylipin transcripts increased and six decreased in abundance in susceptible plants. The transcripts in this category were derived from three types of genes: ω-3 fatty acid desaturase (ωFAD), lipoxygenase (LOX), and allene oxide synthase (AOS). All these enzymes participate in oxylipin synthesis. In each type, there were transcripts with increased abundance in resistant plants.

Among the 27 transcripts putatively involved in wax synthesis, nine increased and four decreased in abundance in resistant plants after HF attack (Table 3). In contrast, four increased and nine decreased in susceptible plants after HF attack. There are many different types of genes involved in synthesis of different types of cuticle wax (Kunst and Samuel 2009) and only a small number of gene transcripts were included in the microarray. Among the different types, transcripts encoding fatty acyl CoA reductases were affected the most. Fatty acyl-CoA reductases catalyze the formation of fatty alcohols, components of plant cuticle wax.

Twelve transcripts encoding fatty acid metabolic enzymes were included in the microarray, with five involved in fatty acid synthesis and seven involved in fatty acid degradation (Table 3). All five transcripts for fatty acid synthesis encoded acetyl-CoA carboxylase, an enzyme required for the irreversible carboxylation of acetyl-CoA to produce malonyl-CoA during fatty acid synthesis. The seven transcripts for fatty acid degradation encoded two types of enzymes: enoyl-CoA hydratase (an enzyme that hydrates the double bond on acyl-CoA) and fatty acid hydroxylase (an enzyme involved in fatty acid

Table 3. Changes in transcript abundance in wheat following Hessian fly (HF) larval attack

			Increased abundance[a]		Decreased abundance[a]	
Functional category[b]	**Time after attack (h)[c]**	**Total probe sets[d]**	**R**	**S**	**R**	**S**
Lipolysis	Total	43	22	5	1	9
	6	43	12	0	1	6
	12	43	17	0	0	6
	24	43	16	5	0	2
	72	43	2	1	0	0
Lipid transport	Total	55	27	0	9	29
	6	55	26	0	6	28
	12	55	22	0	9	28
	24	55	24	0	5	29
	72	55	4	0	0	0
Oxylipin synthesis	Total	31	23	0	8	6
	6	31	22	0	2	1
	12	31	22	0	2	2
	24	31	12	0	6	5
	72	31	1	0	0	0
Cuticle wax synthesis	Total	27	9	4	4	9
	6	27	6	1	3	6
	12	27	6	0	4	9
	24	27	8	4	2	8
	72	27	5	1	0	0
Fatty acid synthesis	Total	5	0	0	0	0
	6	5	0	0	0	0
	12	5	0	0	0	0
	24	5	0	0	0	0
	72	5	0	0	0	0
Fatty acid catabolism	Total	7	0	2	0	0
	6	7	0	2	0	0
	12	7	0	2	0	0
	24	7	0	2	0	0
	72	7	0	2	0	0

[a] Number of transcripts with significant changes in abundance in infested plants compared with that in uninfested control plants. R = resistant plants during incompatible interaction and S = susceptible plants during compatible interaction.

[b] Gene category based on probe-set annotation. Lipolysis, lipases; Lipid transport, lipid transfer proteins; Oxylipin synthesis, fatty acid desaturase, lipoxygenase, and allene oxide synthase; Cuticle wax synthesis, various enzymes; Fatty acid synthesis, acetyl-CoA carboxylase; and Fatty acid catabolism, enoyl-CoA hydratase fatty acid hydroxylase.

[c] Hours after the initial HF larval attack.

[d] Number of probe-sets on Wheat Affymetrix microarray.

degradation). No significant changes in the abundance of all 12 transcripts were observed in resistant plants after HF attack. In contrast, the abundance of the two transcripts encoding fatty acid hydroxylases increased in susceptible plants within 24 h after HF attack.

HF attack increases the concentrations of selected fatty acids and 12-oxo-phytodienoic acid.

The change in gene expression in lipid-related pathways pointed to increased accumulation of defense-related molecules from the mobilized lipids. Because the majority (over 50%) of membrane lipids contained 18 carbon fatty acids, we measured the concentrations of 18 carbon fatty acids in plants during both compatible and incompatible interactions (Fig. 3A). The concentrations of the fatty acid 18:3 increased over four- and twofold in resistant plants during incompatible interactions 24 and 72 h, respectively, after HF infestation. In comparison, the concentration of the fatty acid 18:3 decreased approximately 50% at 24 h, and there was no significant change at 72 h in susceptible plants during compatible interactions. The concentrations of the fatty acid 18:2 did not change significantly in either resistant or susceptible plants. The concentrations of the fatty acid 18:1 increased approximately twofold at both 24 and 72 h after HF infestation in resistant plants but there was no significant change in susceptible plants. The concentration of the fatty acid 18:0 was unmeasurable under our conditions (data not shown).

The concentrations of downstream metabolites 12-oxo-phytodienoic acid (OPDA) and jasmonic acid (JA) were also measured (Fig. 3B). The concentrations of OPDA increased more than 30- and 8-fold at 24 and 72 h, respectively, after HF infestation in resistant plants during incompatible interactions. No significant changes in OPDA concentration were observed in susceptible plants during compatible interactions. Interestingly, no significant changes in JA concentrations were observed in plants during both compatible and incompatible interactions. For comparison, we also measured the concentrations of salicylic acid (SA), another defense-related plant hormone. The amounts of SA increased more than eight- and fivefold at 24 and 72 h, respectively, after HF infestation in resistant plants. No significant changes in SA concentration were observed in susceptible plants.

Exogenous OPDA and SA decrease HF survival in susceptible wheat.

Because both OPDA and SA increased dramatically in resistant plants, we tested whether exogenous application of OPDA to susceptible plant genotypes has an impact on HF larval survival. The survival rate of HF larvae decreased approximately 50% in plants treated with exogenous OPDA (Fig. 3Ca). Similarly, application of SA also resulted in lower survival in comparison with untreated control plants. Interestingly, there was no further increase in HF larval mortality when both OPDA and JA were applied (data not shown). To examine whether the decrease in larval survival was due to any effect of the exogenous chemical application on HF larval hatch or migration, the number of HF larvae in treated plants was counted 24 h after larval hatch and migration. There was no difference in the numbers of larvae that migrated successfully to the feeding site between control and SA-treated plants (Fig. 3Cb). OPDA was omitted from this observation due to the high cost of the chemical.

Epidermal permeability of wheat sheath in resistant plants was not affected by HF attack.

The mobilized membrane lipids could also be converted into cuticular wax and cutin, which can result in strengthened cell walls in resistant plants during incompatible interactions (Kosma et al. 2010). Accordingly, epidermal permeability of the wheat sheath at the HF-feeding site was determined by neutral red staining (Fig. 3D). Wheat sheaths of uninfested plants were not permeable to the red staining dye. Wheat sheaths of susceptible plants during compatible interactions were strongly permeable to the dye, resulting in full red staining. In comparison, wheat sheaths of resistant plants during incompatible interactions were also not permeable to the dye, indicating that either the cell wall was strengthened or damage inflicted by HF was repaired in resistant plants following HF attack.

DISCUSSION

We profiled changes in membrane lipids in HF-feeding tissue of wheat seedlings during incompatible and compatible interactions, and examined changes in transcript abundance of genes involved in lipid metabolism. We observed a rapid reduction of membrane lipids, specifically in resistant plants during incompatible interactions. Along with the reduction in membrane lipids, transcripts encoding various lipases rapidly accumulated in resistant plants (Table 3; Fig. 2), suggesting that lipid mobilization was achieved via lipolysis. Changes in membrane lipids in plants have been observed in response to other stresses, including low temperatures (Cyril et al. 2002; Vigh et al. 1985; Wang et al. 2006; Welti et al. 2002), salts (Surjus and Durand 1996); heavy metals (Nesterov et al. 2008), herbicides (Keppler and Novacky 1989), drought (Monteiro De Paula et al. 1990; Ferrari-Iliou et al. 1984; Gigon et al. 2004), and pathogens (Keppler and Novacky 1989; Saini et al. 1994); and lipolytic enzyme transcript levels and activities are elevated in stressed plants (El-Jafid et al. 1989; Gigon et al. 2004; Shah 2005; Welti et al. 2002).

Previously, we have observed that there was a transient but severe downregulation of the central metabolic pathways, including glycolysis, the citric acid cycle, and the pentose phosphate pathway, specifically in resistant plants during incompatible interactions (Zhu et al. 2008). The downregulation of primary metabolism is likely for the purpose of conserving energy and nutrients for plant defense. The rapid mobilization of membrane lipids could provide necessary resources for the synthesis of defense chemicals. Because the membrane lipids in leaf sheaths of wheat seedlings contained predominantly 18 carbon acyl chains, the accumulation of the 18 carbon fatty acids was analyzed in resistant plants during incompatible interactions after HF attack. The analysis revealed an unbalanced accumulation of these fatty acids, with a dramatic increase in 18:3 and 18:1, no change in 18:2, and undetected 18:0 (Fig. 3A). The impact of the unbalanced accumulation of the fatty acids remains to be determined. Some of the polyunsaturated fatty acids, such as 18:1 and 18:3, are themselves defense-signaling molecules (Shah 2005), in addition to being precursors for oxylipin synthesis. In association with the increase of 18:1 and 18:3 fatty acids, the amounts of OPDA increased greatly in resistant plants (Fig. 3B). Interestingly, the amount of JA did not change in the same plants. The significance of elevated OPDA but not JA again remains to be delineated. OPDA itself does play a role in plant defense (Chandra-Shekara et al. 2007; Stintzi et al. 2001). In addition to elevated OPDA, the defense signaling molecule SA also increased greatly in resistant plants during incompatible interactions. The interactions between JA and SA pathways in plant defense is complex, including both antagonism and synergism (Mur et al. 2006). On the one hand, high levels of SA inhibit endogenous production of JA (Mur et al. 2006). On the other hand, genes in both JA and SA pathways are often induced upon pest infestation

(Zhu-Salzman et al. 2004). In wheat resistance against HF attack, genes in both JA and SA pathways are upregulated (Liu et al. 2007). Our data suggested that the intermediates, including 18:1 and 18:3 fatty acids, and OPDA in the JA synthetic pathway may provide synergism with the SA pathway in wheat defense against HF attack. Consistent with this possibility, exogenous application of both OPDA and SA increased HF larval mortality (Fig. 3C).

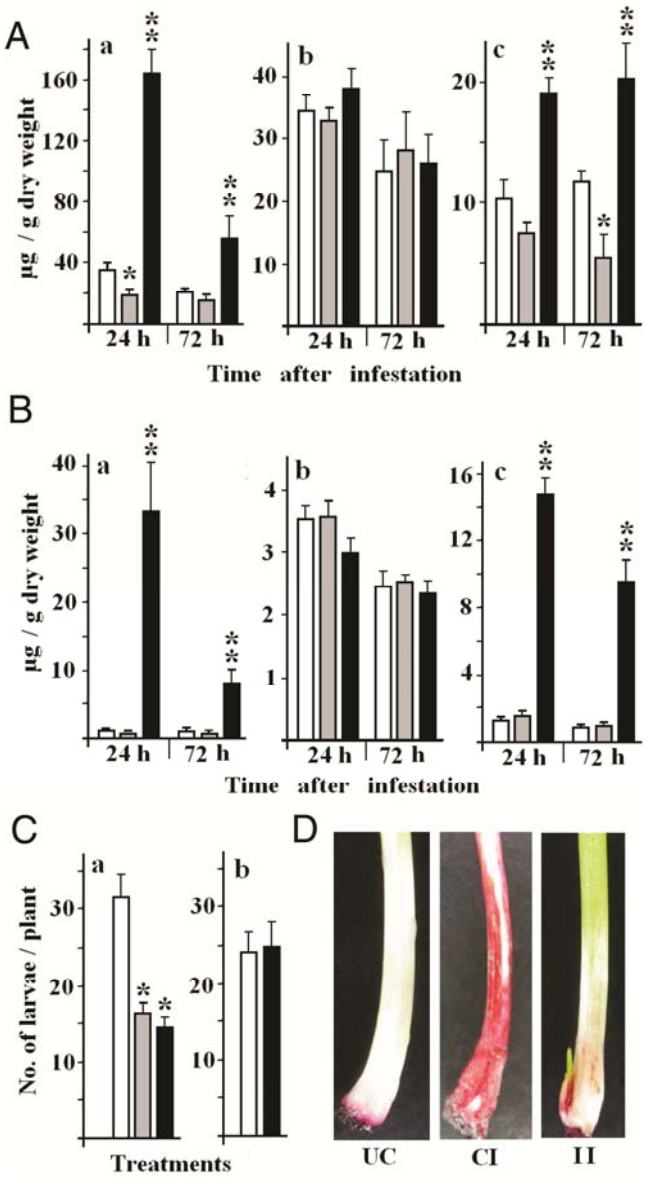

Fig. 3. Analysis of representative downstream metabolites of membrane lipids and the impact of exogenous jasmonic acid (JA), 12-oxo-phytodienoic acid (OPDA), and salicylic acid (SA) on Hessian fly (HF) larval survival. **A,** Changes in concentrations of a, 18:3; b, 18:2; and c, 18:1 fatty acids. White, gray, and black bars represent samples from uninfested plants, plants during compatible interaction, and plants during incompatible interactions, respectively, at 24 or 72 h after HF infestation. **B,** Changes in concentrations of a, OPDA; b, JA; and c, SA. Denotations are the same as in A. **C,** Impact of exogenous OPDA and SA on HF larval migration and survival on plant growth. a, Impact of exogenous OPDA and SA on HF larval survival. White, gray, and black bars represents control, OPDA-treated, and SA-treated plants, respectively. b, No significant impact of exogenous SA application on HF larval migration. White and black bars represent the numbers of larvae that successfully migrated into the feeding site on control and SA-treated plants, respectively. **D,** Epidermal permeability analysis of cell wall. UC, CI, and II represent samples of uninfested plants, plants during compatible interactions, and plants during incompatible interactions, respectively. Samples were collected at 48 h after larval infestation. Single and double asterisks (*) indicate differences significant at *P* values of 0.05 and 0.01, respectively, on Tukey's honestly significant difference test.

The coordinated upregulation of genes in the oxylipin synthesis pathway, including genes coding for key enzymes such as ωFAD and AOS, was likely the molecular basis for conversion of mobilized membrane lipids into signaling defense molecules such as 18:1 and 18:3 fatty acids and OPDA. By the same logic, part of the mobilized lipids might have also been converted into cuticular wax and cutin (Kosma et al. 2010). The abundance of several transcripts encoding enzymes potentially involved in wax and cutin synthesis increased in resistant plants (Table 3). Increased abundance of many wax-related transcripts was also reported by another group (Kosma et al. 2010). Other evidence supporting wax synthesis came from the strong upregulation of many transcripts encoding different lipid-transfer proteins. Analysis of amino acid sequences encoded by corresponding rice genes that shared the highest similarity with the increased wheat transcripts revealed that all except one have a secretion signal peptide, even though the protein sequences themselves are very diversified (data not shown). Lipid-transfer proteins with a secretion signal are likely secreted into the cell wall and participate in wax and cutin formation (Kader 1996). Consistent with the possibility of increased wax and cutin, the ability of HF larvae to compromise wheat sheath impermeability is neutralized in resistant plants during incompatible interactions (Fig. 3D).

Although the evidence is solid for the conversion of mobilized membrane lipids into defense molecules in resistant plants, other questions remain to be answered. For example, does increased mobilization of membrane lipids affect membrane integrity? The decrease in lipid concentration appeared to be relatively similar among different classes, except PA and lysophospholipids (Table 2), which are the degradation products of other phospholipids. Increases in the levels of these classes can increase membrane instability (Lee and Chan 1977). On the other hand, considering that HF attack causes a growth deficit in resistant plants during the first few days (Anderson and Harris 2006), the mobilized membrane lipids might be compensated by a reduced amount of total membrane in temporally nondividing cells. Among the upregulated lipase transcripts, the majority of them encoded lipase 3 and GDSL-motif lipases. Phospholipase transcripts were less upregulated and, in some cases, upregulation could only be detected by the more sensitive method qPCR (Fig. 2). The strong increase in non-phospholipase transcripts suggested that other non-polar lipids such as triacylglyerides were also mobilized. The mobilization of structural components and nutrients might constitute a defense cost, resulting in a growth deficit during this period. Mobilization of membrane lipids for defense observed in this study is also likely to happen in plants against some biotrophic pathogens and other herbivores. Various phospholipases are upregulated in plants in response to pathogen attack (Dhondt et al. 2000; de Torres Zabela et al. 2002; Dhondt et al. 2000; Shah 2005). Many other lipid-metabolism-related genes are also upregulated in other plant–pathogen interactions and the upregulation is often associated with increased plant resistance (Kachroo et al. 2001; Kwon et al. 2009; Maldonado et al. 2002; Oh et al. 2005; Ohta et al. 1991; Sarowar et al. 2009; Shah 2005).

In conclusion, our data, along with published evidence, suggested that membrane lipids are rapidly mobilized to produce defense molecules that enhance wheat resistance to HF. Specifically, we think membrane lipids were mobilized by lipolysis (Fig. 4). The released fatty acids from mobilized membrane lipids were then converted into defense signaling molecules

such as 18:3 and 18:1 fatty acids and OPDA and, potentially, into cell-wall-strengthening components, including wax and cutin. The rapid mobilization of membrane lipids and subsequent conversion into defense molecules were likely part of a wheat defense mechanism against HF attack during incompatible interactions. On the other hand, the inhibition of both lipid mobilization and lipid conversion into defense molecules might be a necessary molecular process for the susceptibility of wheat seedlings during compatible interactions with HF.

MATERIALS AND METHODS

Plant infestation and sample collection.

Two wheat isogenic lines, Newton and Molly, were used in this study. Newton is a susceptible cultivar whereas Molly is a backcrossed offspring containing the *R* gene *H13* (Patterson et al. 1994). A Kansas HF population that is virulent to Newton but avirulent to Molly was used for infestation (Chen et al. 2009a). The Kansas population contains the *Avr* gene corresponding to the R gene *H13*.

Twenty germinated wheat seeds were planted in each 10-cm-diameter pot filled with PRO-MIX 'BX' potting mix (Hummert Inc., Earth City, MO, U.S.A.) in a growth chamber programmed at 20 and 18°C (light and darkness, respectively) with a photoperiod of 14 and 10 h (light and darkness, respectively). When wheat seedlings reached the 1.5-leaf stage (stage 11 on Zadoks scale) (Zadoks et al. 1974), the plants were infested with one HF female per plant for 6 h, when the egg density reached approximately 25 eggs per plant. The females were then removed from the plants to prevent them from laying more eggs. In this way, the larval hatching time was relatively uniform. The initial HF larval attack time was determined as follows. Three days after the eggs were laid, a few plants were cut at the base (just above the node where roots begin). The cut plants were put into a 50-ml plastic tube with 5 ml of water. The tubes with the plants were put in the same growth chamber and monitored for larval hatch every 10 min. The time at which approximately half of the expected neonates (approximately 10 per plant) from the cut plants entered into the water was taken as the HF larval initial attack time. Each treatment was biologically repeated four times for lipid profiling but three times for qPCR. Each sample was collected from 20 individual plants in a pot. For each treatment, control plants were grown in the same growth chamber under identical conditions. Plant samples were collected by obtaining a 10-mm-long section of the second leaf sheath after removing HF larvae. Samples for RNA extraction were frozen in liquid nitrogen (N_2) immediately after sampling and stored at –80°C until RNA extraction. Samples

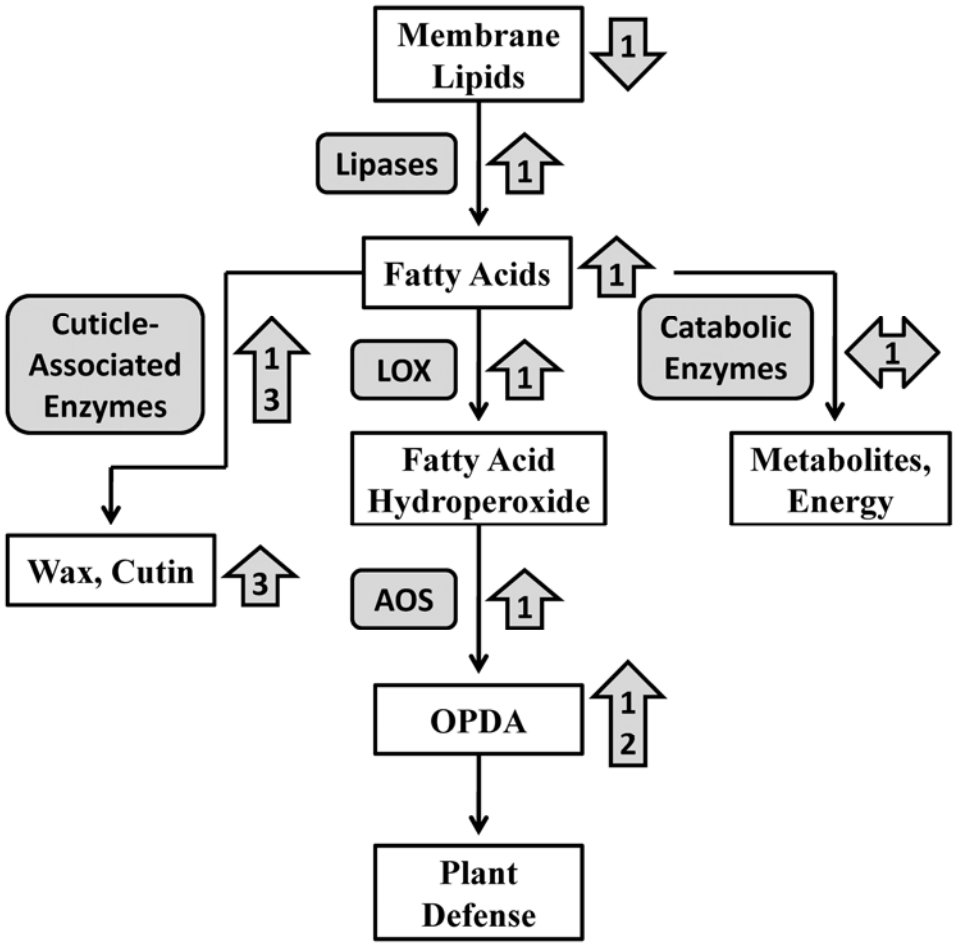

Fig. 4. Model for mobilization and conversion of lipids into defense molecules in resistant plants during incompatible interactions. White rectangles indicate chemicals, plant products, and functions. Gray rectangles indicate enzymes that accelerate the chemical flow. Upright arrows indicate increases in either the abundance of transcripts or the concentrations of chemicals. The horizontal arrow indicates no change. Numbers in the arrows indicate the source of evidence for the indicated changes, with 1 from this study, 2 from Zhu and associates (2010), and 3 from Kosma and associates (2010). LOX and AOS represent lipoxogenases and allene oxide synthases, respectively. The model shows that lipids were mobilized by elevated lipases to release fatty acids. Some of the released fatty acids were converted into oxylipins via fatty acid hydroperoxides; this notion is supported by a strong increase in the abundance of oxylipin-related transcripts and an elevation of 12-oxo-phytodienoic acid (OPDA). Some of the released fatty acids were converted into components of wax and cutin, supported by an increase in the abundance of transcripts encoding cuticle-associated enzymes and an elevation in the level of wax components.

for lipid extraction were immediately soaked in 3 ml of preheated (75°C) isopropanol with 0.01% butylated hydroxytoluene (BHT) in a 50-ml tube with a Teflon-lined screw cap for 15 min to inactivate phospholipase D. To each tube with samples was then added 1.5 ml of chloroform and 0.6 ml of water, followed by a brief vortex. The samples were then stored in a –20°C freezer.

Lipid extraction.

Lipids were extracted from tissue samples following the method described by Welti and associates (2002). Briefly, lipids were extracted from the frozen wheat tissues by shaking at room temperature for 1 h. The lipid extract was then transferred to a glass tube with Teflon-lined screw-cap, and 4 ml chloroform/methanol (2:1, vol/vol) with 0.01% BHT was added to the remaining tissues. The sample tissues were again shaken at room temperature for 30 min. The extract from the second extraction was combined with the first extract. This extraction procedure was repeated five times, at which time the wheat tissues were completely white. The combined extracts were washed once with 1 ml of 1 M KCl and once with 2 ml of water. After washing, tubes with lipid samples were filled with nitrogen to allow the solvent to evaporate. Lipid extract was then dissolved in 1 ml of chloroform for further analysis. The remaining plant tissues after lipid extraction were dried at 105°C overnight. The dried tissues were weighed and recorded as "dry mass" for each sample.

ESI tandem mass spectrometry analysis.

To profile lipid species and determine lipid concentration in individual samples, an automated ESI tandem mass spectrometry approach was followed as described by Welti and associates (2002), with modifications as described by Xiao and associates (2010). The apparent lipid molecular species were quantified in comparison with internal standards of the same lipid class. Analysis of free fatty acids, OPDA, JA, and SA was conducted as described by Zhu and associates (2010).

Microarray data collection and analysis.

The Affymetrix GeneChip Wheat Genome Array (Affymetrix Inc., Santa Clara, CA, U.S.A.) was used for wheat microarray studies. RNA was isolated from frozen tissue with TRIzol reagent (Invitrogen, Carlsbad, CA, U.S.A.), according to the manufacturer's instruction. Generation of cRNA, fluorescent labeling, hybridization, scanning, and quantification of hybridized Affymetrix wheat arrays were performed according to the Affymetrix GeneChip Expression Analysis Technical Manual (Affymetrix Inc.) in the Kansas State University Gene Expression Facility (Liu et al. 2007).

Three biological repeats were conducted for each treatment. Microarray data were initially processed using GeneChip Robust Multiarray Averaging (Wu et al. 2004), an improved version of robust multiarray averaging. Probe sets that are potentially involved in lipid metabolism were analyzed with the empirical Bayes method, followed by the Benjamini and Hochberg (1995) multiple comparison-adjustment method to control false discovery rate. Microarray data were deposited to the database of National Center for Biotechnology Information with accession number GSE34445.

qPCR analysis.

DNase-treated total RNA was used for cDNA synthesis using random hexamers with a superscript reverse-transcriptase kit (BD Biosciences, San Jose, CA, U.S.A.). Samples were then treated with RNase H (Invitrogen). cDNA was quantified on a Nanodrop ND-1000 (NanoDrop Technologies Inc., Wilmington, DE, U.S.A.) spectrophotometer and samples were diluted to 15 ng/µl to ensure equal amounts of cDNA template for quantification of mRNA abundance.

qPCR was performed with iQ SYBR Green Supermix on an iCycler real time detection system (Bio-Rad, Hercules, CA, U.S.A.). Each reaction was carried out with 2 µl of a 1/40 (vol/vol) dilution of the first cDNA strand and 0.5 µM each primer in a total volume of 25 µl. The cycling conditions were 95°C for 5 min followed by 45 cycles of denaturation at 95°C for 20 s and annealing and extension at 62 to 64.5°C, depending on the primer set, for 45 s. At the end of the cycles, PCR amplification specificity was verified by obtaining a dissociation curve, derived by cooling the denatured samples to 55°C and raising the temperature 0.5°C for 10 s for each cycle for a total of 80 cycles, until reaching 95°C. The PCR products were analyzed on 1.5% agarose gels and, subsequently, purified and sequenced to confirm faithful amplification. Primers were designed using the Beacon Designer (v2.0) software from Biosoft International (Palo Alto, CA, U.S.A.) and the primer sequences were listed in Table 1. Plasmid DNA containing the corresponding insert was used to generate a calibration standard curve, where cycle threshold values are plotted to serve as standard concentrations. The transcript concentration for each sample was calculated based on the standard concentrations. A negative control without template was always included for each primer set. Template concentrations in different samples were normalized by the ribosomal protein L21 (*RPL21*).

Analysis of exogenous application of OPDA and SA on HF larval survival.

Solutions of OPDA or SA (3 mM) in 0.02% Silwet and 0.5% ethanol were sprayed on wheat seedlings with a small hand sprayer. First spray was carried out 24 h before HF larval hatch. The spray was continued once every 24 h for five consecutive days. The numbers of surviving larvae were counted 3 weeks after larval hatch. To determine whether the first spray affected larval hatch and migration, a set of control and treated plants was dissected, and neonate larvae that reached the feeding site were counted and compared 24 h after larval hatch.

Epidermal cell permeability staining.

Neutral red stain (Sigma-Aldrich, St. Louis) was used to determine epidermal permeability of cells as described by Kosma and associates (2010). Briefly, plants were dissected 2 days after the initial HF larval attack. After peeling off the first leaf sheath, the HF larval feeding site of the second leaf sheath was stained with 0.1% neutral red stain for 10 min, followed by washing five times with water. Uninfested plants were dissected and stained as negative controls in the same way. After staining, plant tissues were examined under a fluorescent microscope (Zeiss Axioplan-2) and photographed with a Nikon Coolpix 4500 digital camera.

Statistical analysis.

For comparison of total lipids, analysis of variance (ANOVA) was first conducted, followed by the Tukey's honestly significant difference test to identify significant differences between uninfested and infested samples for each genotype at each timepoint. These tests ensured that the family-wise type I error for all comparisons was controlled at the desired significance level.

For comparison of lipid subgroups, the empirical Bayes procedure was used to determine whether a change in percentage was significant, followed by Bonferroni correction for multiple adjustments. Hierarchical linear models were applied to evaluate how the concentration of each lipid subgroup varied between uninfested and infested samples at each timepoint. *P* values from a moderated *F* statistic were multiplied by the

number of lipid subgroups as well as the number of timepoints (truncated at 1).

For comparison of individual lipid species, the empirical Bayes method of Smyth (2004), with moderated *t* statistics, was used to compare data from uninfested and infested wheat seedlings of each plant genotype at each timepoint. Bonferroni correction was applied to control the family-wise error rate for each lipid species.

For qPCR data, statistical significance was analyzed by ANOVA using the PROC MIXED procedure of SAS (SAS/STAT User's Guide, version 9.13; SAS Institute Inc., Cary, NC, U.S.A.). The ANOVA model included treatment, timepoints, and their interaction. Data from three biological replicates (each replicate assayed two times in independent qPCR experiments) were combined and included as a random effect in the analysis. Fold-change calculations were performed by comparing mRNA abundance of selected genes in infested plants with that of the same genes in uninfested controls. Fold change was considered statistically significant if the P value was ≤ 0.05. The number of fold increase or decrease was defined as the number of fold change minus 1; for example, a twofold change was considered onefold increase or decrease.

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LITERATURE CITED

Anderson, K. G., and Harris, M. O. 2006. Does *R* gene resistance allow wheat to prevent plant growth effects associated with Hessian fly (Diptera: Cecidomyiidae) attack? J. Econ. Entomol. 99:1842-1853

Behura, S. K., Valicente, F. H., Rider, S. D., Chen, M. S., Jackson, S., and Stuart, J. J. 2004. A physically anchored genetic map and linkage to avirulence reveals recombination suppression over proximal region of Hessian fly chromosome A2. Genetics 167:343-355.

Benjamini, Y., and Hochberg, Y. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B. 57:289-300.

Bent, A. F., and Mackey, D. 2007. Elicitors, effectors, and *R* genes: The new paradigm and a lifetime supply of questions. Annu. Rev. Phytopathol. 45:399-436.

Byers, R. A., and Gallun, R. L. 1971. Ability of the Hessian fly to stunt winter wheat: Effect of larval feeding on elongation of leaves. J. Econ. Entomol. 65:955-958.

Chandra-Shekara, A. C., Venugopa, S. C., Barman, S. R., Kachroo, A., and Kachroo, P. 2007. Plastidial fatty acid levels regulate resistance gene-dependent defense signaling in *Arabidopsis*. Proc. Natl. Acad. Sci. U.S.A. 104:7277-7252.

Chen, M. S., Zhao, H. X., Zhu, Y. C., Scheffler, B., Liu, X. M., Liu, X., Hulbert, S., and Stuart, J. J. 2008. Analysis of transcripts and proteins expressed in the salivary glands of Hessian fly (*Mayetiola destructor*) larvae. J. Insect Physiol. 54:1-16.

Chen, M. S., Echegaray, E., Whitworth, R. J., Wang, H., Sloderbeck, P. E., Knutson, A., and Giles, K. L. 2009a. Virulence analysis of Hessian fly (*Mayetiola destructor*) populations from Texas, Oklahoma, and Kansas. J. Econ. Entomol. 102:774-780.

Chen, M. S., Liu, X. M., Wang, H., and El Bouhssini, M. 2009b. Hessian fly (*Mayetiola destructor*) interactions with barley, rice, and wheat seedlings. J. Econ. Entomol. 102:1663-1672.

Chen, M. S., Liu, X. M., Yang, Z., Zhao, H., Shukle, R. H., Stuart, J. J., and Hulbert, S. 2010. Unconventional conservation among genes encoding small secreted salivary gland proteins from a gall midge. BMC Evol. Biol. 10:296.

Cyril, J., Powell, G. L., Duncan, R. R., and Baird, W. V. 2002. Changes in membrane polar lipid fatty acids of seashore paspalum in response to low temperature exposure. *Crop Sci.* 42:2031-2037.

de Torres Zabela, M., Fernandez-Delmond, I., Niittyla, T., Sanchez, P., and Grant, M. 2002. Differential expression of genes encoding *Arabidopsis* phospholipases after challenge with virulent or avirulent *Pseudomonas* isolates. Mol. Plant-Microbe Interact. 15:808-816.

Dhondt, S., Geoffroy, P., Stelmach, B. A., Legrand, M., and Heitz, T. 2000. Soluble phopholipase A2 activity is induced before microemulsion accumulation in tobacco mosaic virus-infested tobacco leaves and is contributed by patatin-like enzymes. Plant J. 23:431-440.

El-Jafid, L., Pham-thi, A. T., Zuily-Fodl, Y., and Vieira da Silva, J. 1989. Enzymatic breakdown of polar lipids in cotton leaves under water stress. I. Degradation of monogalactosyl-diacylglyceral. Plant Physiol. Biochem. 27:495-520.

Ferrari-Iliou, R., Pham-thi, A. T., and Vieira da Silva, J. 1984. Effects of water stress on the lipid and fatty acid composition of cotton (*Gossypium hirsutum*) chloroplasts. Physiol. Plant. 62:219-224.

Forrester, J. S., Milne, S. B., Ivanova, P. T., and Brown, H. A. 2004. Computational lipidomics: A multiplexed analysis of dynamic changes in membrane lipid composition during signal transduction. Mol. Pharmacol. 65:813-821.

Gigon, A., Matos, A.-R., Laffray, D., Zuily-fodil, Y., and Pham-thi, A.-T. 2004. Effect of drought stress on lipid metabolism in the leaves of *Arabidopsis thaliana* (ecotype Columbia). Ann. Bot. 94:345-351.

Harris, M. O., Stuart, J. J., Mohan, M., Nair, S., Lambe, R. J., and Rohfritsch, O. 2003. Grasses and gall midges: Plant defense and insect adaptation. Annu. Rev. Entomol. 48:549-557.

Harris, M. O., Freeman, T. P., Rohfritsch, O., Anderson, K. G., Payne, S. A., and Moore, J. A. 2006. Virulent Hessian fly (Diptera: Cecidomyiidae) larvae induce a nutritive tissue during compatible interactions with wheat. Annu. Rev. Entomol. 99:305-316.

Harris, M. O., Freeman, T. P., Moore, J. A., Anderson, K. G. Payne, S. A., Anderson, K. M., and Rohfritsch, O. 2010. H-Gene-mediated resistance to Hessian fly exhibits features of penetration resistance to fungi. Phytopathology 100:279-89.

Hatchett, J. H., and Gallun, R. L. 1970. Genetics of the ability of the Hessian fly, *Mayetiola destructor*, to survive on wheat having different genes for resistance. Ann. Entomol. Soc. Am. 63:1400-1407.

Hatchett, J. H., Kreitner, G. L., and Elzinga, R. J. 1990. Larval mouthparts and feeding mechanism of the Hessian fly (Diptera: Cecidomyiidae). Ann. Entomol. Soc. Am. 83:1137-1147.

Hueck, C. J. 1998. Type III protein secretion systems in bacterial pathogens of animals and plants. Microbiol. Mol. Biol. Rev. 62:379-433.

Kachroo, P., Shanklin, J., Shah, J., Whittle, E. J., and Klessig, D. 2001. A fatty acid desaturase modulates the activation of defense signaling pathways in plants. Proc. Natl. Acad. Sci. U.S.A. 98:9448-9453.

Kader, J.-C. 1996. Lipid-transfer proteins in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 47:627-645.

Keen, N. T. 1990. Gene-for-gene complementarity in plant-pathogen interactions. Annu. Rev. Genet. 24:447-463.

Keppler, L. D., and Novacky, A. 1989. Changes in cucumber cotyledon membrane lipid fatty acids during paraquat treatment and a bacteria-induced hypersensitive reaction. Phytopathology 79:705-708.

Kosma, D. K., Nemacheck, J. A., Jenks, M. A., and Williams, C. E. 2010. Changes in properties of wheat leaf cuticle during interactions with Hessian fly. Plant J. 63:31-43.

Kunst, L., and Samuels, L. 2009. Plant cuticles shine: Advances in wax biosynthesis and export. Curr. Opin. Plant Biol. 12:721-727.

Kwon, S. J., Jin, H. C., Lee, S., Nam, M. H., Chung, J. H., Kwon, S., Ryu, C.-M., and Park, O. K. 2009. GDSL lipase-like 1 regulates systemic resistance associated with ethylene signaling in *Arabidopsis*. Plant J. 58:235-245.

Laxalt, A. M., and Munnik, T. 2002. Phospholipid signaling in plant defense. Curr. Opin. Plant Biol. 5:332-338.

Lee, Y., and Chan, S. I. 1977. Effect of lysolecithin on the structure and permeability of lecithin bilayer vesicles. Biochemistry 16:1303-1309.

Liu, X. L., Bai, J., Huang, L., Zhu, L., Liu, X., Weng, N., Reese, J. C., Harris, M., Stuart, J. J., and Chen, M. S. 2007. Gene expression of different wheat genotypes during attack by virulent and avirulent Hessian fly (*Mayetiola destructor*) larvae. J. Chem. Ecol. 33:2171-2194.

Liu, X. M., Brown-Guedira, G. L., Hatchett, J. H., Owuoche, J. O., and Chen, M. S. 2005. Genetic characterization and molecular mapping of a Hessian fly-resistance gene transferred from *T. turgidum* ssp. *dicoccum* to common wheat. Theor. Appl. Genet. 111:1308-1315.

Liu, X. M., Williams, C. E., Nemacheck, J. A., Wang, H. Y., Subramanyam, S., Zheng, C., and Chen, M. S. 2010. Reactive oxygen species are involved in plant defense against a gall midge. Plant Physiol. 152:985-999.

Maldonado, A. M., Doerner, P., Dixon, R. A., Lamb, C. J., and Cameron, R. K. 2002. A putative lipid transfer protein involved in systemic resistance signaling in *Arabidopsis*. Nature 419:399-403.
McMahon, H. T., and Gallop, J. L. 2005. Membrane curvature and mechanisms of dynamic cell membrane remodeling. Nature 438:590-596.
Monteiro De Paula, F., Pham-thi, A. T., Vieira De Silva, J., Justin, A. M., Demandre, C., and Mazliak, P. 1990. Effects of water stress on the molecular species composition of polar lipids from *Vigna unguiculata* L. leaves. Plant Sci. 66:185-193.
Mur, L. A., Kenton, P., Atzorn, R., Miersch, O., and Wasternack, C. 2006. The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. Plant Physiol. 140:249-262.
Nesterov, V. N., Rozentsvet, A., and Murzaeva, V. 2008. Changes in lipid composition in the tissues of fresh-water plants *Hydrilla verticillata* induced by accumulation and elimination of heavy metals. Russ. J. Plant Physiol. 56:85-93.
Oh, I. S., Park, A. R., Bae, M. S., Kwon, S. J., Kim, Y. S., Lee, J. E., Kang, N. Y., Lee, S., Cheong, H., and Park, O. K. 2005. Secretome analysis reveals an *Arabidopsis* lipase involved in defense against *Alternaria brassicicola*. Plant Cell 17:2832-2847.
Ohta, H., Shida, K., Peng, Y.-L., Furusawa, I., Shishiyama, J., Aibara, S., and Morita, Y. 1991. A lipoxygenase pathway is activated in rice after infection with the rice blast fungus *Magnaporthe grisea*. Plant Physiol. 97:94-98.
Patterson, F. L., Mass, F. B., III, Foster, J. E., Ratcliffe, R. H., Cambron, S., Safranski, G., Taylor, P. L., and Ohm, H. W. 1994. Registration of eight Hessian fly resistant common winter wheat germplasm lines (Carol, Erin, Flynn, Iris, Joy, Karen, Lola, and Molly). Crop Sci. 34:315-316.
Saini, R. S., Chawla, H. K. L., and Wagle, D. S. 1994. Fatty acids compositions of polar and non-polar lipid fractions of wheat leaves inoculated with three brown rust races during progressive pathogenesis. Biol. Plant 36:469-475.
Sarowar, S., Kim, Y. J., Kim, K. D., Hwang, B. K., Ok, S. H., and Shin, J. S. 2009. Overexpression of lipid transfer protein (LTP) genes enhances resistance to plant pathogens and LTP functions in long-distance systemic signaling in tobacco. Plant Cell Rep. 28:419-427.
Seigler, D. S. 1983. Role of lipids in plant resistance to insects. Pages 303-327 in: Plant Resistance to Insects. ACS Symposium Series, Vol. 208. P. A. Hedin, eds. American Chemical Society, Washington, DC.
Shah, J. 2005. Lipids, lipases, and lipid-modifying enzymes in plant disease resistance. Annu. Rev. Phytopathol. 43:229-260.
Smith, C. M., Liu, X. M., Wang, L. J., Liu, X., Chen, M. S., Starkey, S., and Bai, J. F. 2010. Aphid feeding activates expression of a transcriptome of oxylipin-based defense signals in wheat involved in resistance to herbivory. J. Chem. Ecol. 36:260-276.
Smyth, G. K. 2004. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Stat. Appl. Genet. Mol. Biol. 3:3.
Stintzi, A., Weber, H., Remond, P., Brows, J., and Farmer, E. E. 2001. Plant defense in the absence of jasmonic acid: The role of cyclopetenones. Proc. Natl. Acad. Sci. U.S.A. 98:12837-12842.
Stuart, J. J., Chen, M. S., and Harris, M. O. 2008. The Hessian fly. Pages 93-100 in: Genome Mapping and Genomics in Animals, Volume 1: Genome Mapping and Genomics in Arthropods. W. Hunter and C. Kole, eds. Springer, Berlin, Heidelberg, New York.
Subramanyam, S., Smith, D. F., Clemens, J. C., Webb, M. A., Sardesai, N., and Williams, C. E. 2008. Functional characterization of HFR1, a high-mannose N-glycan-specifc wheat lectin induced by Hessian fly larvae. Plant Physiol. 147:1412-1426.
Surjus, A., and Durand, M. 1996. Lipid changes in soybean root membranes in response to salt treatment. J. Exp. Bot. 47:17-23.
Vigh, L., Horbath, I., Van Hasselt, P. R., and Kuiper, J. C. 1985. Effect of frost hardening on lipid and fatty acid composition of chloroplast thylakoid membranes in two wheat varieties of contrasting hardiness. Plant Physiol. 79:756-759.
Wang, X., Li, W., Li, M., and Welti, R. 2006. Profiling lipid changes in plant response to low temperature. Physiol. Plant. 126:90-96.
Welti, R., Li, W., Li, M., Sang, Y., Biesiada, H., Zhou, H.-E., Rajashekar, C. B., Williams, T. D., and Wang, X. 2002. Profiling membrane lipids in plant stress responses—role of phospholipase Dα in freezing-induced lipid changes in *Arabidopsis*. J. Biol. Chem. 277:31994-32002.
Wu, J. X., Liu, X. M., Zhang, S. Z., Zhu, Y. C., Whitworth, R. J., and Chen, M. S. 2008. Differential responses of wheat inhibitor-like genes to Hessian fly, *Mayetiola destructor*, attacks during compatible and incompatible interactions. J. Chem. Ecol. 34:1005-1012.
Wu, Z., Irizarry, R. A., Gentleman, R., Martinez-Murillo, F., and Spencer, F. 2004. A model-based background adjustment for oligonucleotide expression arrays. J. Am. Stat. Assoc. 99:909-917.
Xiao, S., Gao, W., Chen, Q.-F., Chan, S.-W., Zheng, S.-X., Ma, J., Wang, M., Welti, R., and Chen M.-L. 2010. Overexpression of *Arabidopsis* acyl-coA binding protein ACBP3 promotes starvation-induced and age-dependent leaf senescence. Plant Cell 22:1463-1482.
Zadoks, J. C., Chang, T. T., and Konzak, C. F. 1974. A decimal code for the growth stage of cereals. Weed Res. 14:415-421.
Zhu, L., Liu, X. M., Liu, X., Jeannotte, R., Reese, J. C., Harris, M., Stuart, J. J., and Chen, M .S. 2008. Hessian fly (*Mayetiola destructor*) attack causes dramatic shift in carbon and nitrogen metabolism in wheat. Mol. Plant-Microbe Interact. 21:70-78.
Zhu, L., Liu, X., and Chen, M. S. 2010. Differential accumulation of phytohormones in wheat seedlings attacked by avirulent and virulent larvae. J. Econ. Entomol. 103:178-185.
Zhu-Salzman, K., Salzman, R. A., Ahn, J., and Koiwa, H. 2004. Transcriptional regulation of sorghum defense determinants against a phloem-feeding aphid. Plant Physiol. 134:420-431.

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