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Impact of Transient Heat Stress on Polar Lipid Metabolism in Seedlings of Wheat Near-Isogenic Lines Contrasting in Resistance to Hessian Fly (Cecidomyiidae) Infestation

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ABSTRACT Transient heat stress compromises resistance of host plants to Hessian fly, *Mayetiola destructor* (Say), and other biotic stresses. However, the mechanism for the loss of plant resistance under heat stress remains to be determined. In this study, we determined polar lipid profiles in control and Hessian fly-infested resistant and susceptible wheat seedlings with and without heat stress using an automated electrospray ionization tandem mass spectrometry analysis. Heat stress, alone or in combination with Hessian fly infestation, caused significant reduction in the abundance of total detected polar lipids and double bond index. Changes in lipid profiles in 'Molly' were similar to those in 'Newton' under heat stress. However, changes in lipid profiles in Molly were significantly different from those in Newton following Hessian fly infestation. The combination of heat stress and Hessian fly infestation resulted in unique lipid profiles in comparison with those in plants either treated with heat stress or infested with Hessian fly alone. In addition, a greater impact on lipid metabolism was observed in heat-stressed plants infested with Hessian fly than that in plants treated with either heat stress or Hessian fly alone. Our results suggest that changes in lipid metabolism caused by heat stress may be part of the metabolic pathways through which heat stress suppresses resistance of wheat plants to Hessian fly infestation.

KEY WORDS heat stress, infestation, lipid metabolism, wheat, Hessian fly

Plants are constantly subject to environmental stresses and have evolved mechanisms to adapt (Garrett et al. 2006). Heat stress, even for a short period of time, may significantly alter metabolism of plants, which in turn affect responses of plants to biotic and abiotic stresses (Liu et al. 2013, Currie et al. 2014). In plant–pathogen interactions, plants often exhibit reduced resistance under elevated temperatures (Whitham et al. 1996, Wang et al. 2009), while in plant–insect interactions, elevated temperatures may either decrease or increase plant resistance. For example, the resistance of alfalfa varieties to spotted alfalfa aphids was reduced at 29°C compared with that at 13°C (Isaak et al. 1963), whereas in sorghum, expressions of tolerance and antixenosis to greenbug biotypes C and E are greater at 30°C than at 26° (Thindwa and Teetes 1994).

The Hessian fly, *Mayetiola destructor* (Say), is one of the most destructive pests of wheat, *Triticum aestivium* L., in North America and North Africa (Berzonsky et al. 2003). The insect interacts with wheat in similar ways to pathogen–plant interactions, such as a gene-for-gene relationship between Hessian fly and wheat (Hatchett and Gallun 1970, Stuart et al. 2012). The Hessian fly pest is mainly controlled by development and deployment of resistant wheat cultivars with major dominant resistance genes (Buntin et al. 1992, Garcés-Carrera et al. 2014). However, resistance in wheat cultivars is generally short-lived and lasts for only 6–8 yr once a cultivar with a specific resistance gene is deployed in the field (Gould 1998, Ratcliffe et al. 2000). In addition, environmental conditions such as temperature and humidity also profoundly affect wheat resistance to Hessian fly infestation. Most, if not all, Hessian fly resistance genes (*R* genes) in wheat are temperature-sensitive, i.e., resistance in wheat plants is reduced or lost when temperature is above a certain degree (Tyler and Hatchett 1983, Buntin et al. 1990, Liu et al. 2013, Currie et al. 2014). Molecular analysis revealed that the induction of a gene encoding a heat shock protein is involved in the loss of wheat resistance to Hessian fly under elevated temperatures (Liu et al. 2013). Further analysis is needed to elucidate changes in metabolic pathways that are critical to the loss of wheat resistance to Hessian fly.

Because polar lipids are major components of cell membranes, which function as mechanical barriers separating a cell or its organelles from environments

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Table 1. Treatments applied in the experiment

No. treatment	Lines	Heat stress	Hessian fly infestation
1	Molly	No	No
2	Molly	Yes	No
3	Molly	No	Yes
4	Molly	Yes	Yes
5	Newton	No	No
6	Newton	Yes	No
7	Newton	No	Yes
8	Newton	Yes	Yes

(Van Meer et al. 2008), they are critical interfaces for interactions between plant hosts and abiotic and biotic stresses. Cell membranes consist of diverse lipids, and the composition of lipids changes in responses to internal or external stimuli (McMahon and Gallop 2005). Previous studies have revealed that the composition of polar lipids are affected by many stress conditions, including drought (Gigon et al. 2004), heat (Su et al. 2009), low temperatures (Welti et al. 2002), and parasitic attacks (Zhu et al. 2012). During plants' interaction with pathogens and insects, changes in membranes lipids of host plants could be critical for the synthesis and transduction of defense signaling molecules (Laxalt and Munnik 2002) and the launch of direct or indirect defense responses (Kosma et al. 2010).

We are interested in studying the molecular mechanisms underlining the heat-induced loss of wheat resistance to Hessian fly infestation. When attacked by Hessian fly larvae, wheat plants in incompatible interactions rapidly degrade large portions of lipids and other metabolites, and accumulate increased amounts of compounds related to plant defense, such as salicylic acid and 12-oxo-phytodienoic acid (OPDA; Zhu et al. 2008, 2010, 2012). In a previous study, we observed that 'Molly' seedlings treated with heat stress of 40°C for 6 h exhibited compromised resistance to an avirulent Hessian fly biotype (Currie et al. 2014). The objective of this study is to investigate changes in lipid metabolism in resistant and susceptible wheat plants under heat stress, Hessian fly infestation, and the combination of heat stress and Hessian fly infestation.

Materials and Methods

Plant and Insect Materials. Wheat lines Molly and 'Newton' were used in the study. Molly contains *R* gene *H13* and is a near-isogenic line (NIL) derived from the Hessian fly-susceptible Newton through backcrossing (Patterson et al. 1994). The Hessian fly population called "White eye" was used to infest wheat seedlings. The White eye population is avirulent to Molly at room temperature or below, but virulent to Newton under the same condition (Shukle and Stuart 1993).

Experimental Treatment and Design. Eight treatments were applied in the experiment (Table 1). Molly and Newton plants without heat stress and infestation were used as controls. The experiment was conducted in a growth chamber and arranged as randomized complete block design with five biological replicates.

Plant Preparation and Infestation. Fifteen pregerminated seeds of Molly and Newton were planted in each pot of 10 cm in diameter. The pots were placed in the first growth chamber programmed at 20°C and a photoperiod of 14:10 (L:D) h. Plants that significantly lagged in development were removed from the pots before infestation, resulting in 10–12 plants in each pot for the experiment. At 1.5 leaf stage, approximately eight mated female Hessian fly adults were transferred onto plants confined within a cage with screen on the top. These flies immediately laid eggs on wheat leaves. After eggs were hatched in ≈72 h, larvae crawled down to the bottom of plants, established a feeding site between the first and the second leaf sheaths, and fed on the second leaf sheath. To determine the time when Hessian fly larvae initiated attacks in the plants, an additional set of infested plants under the same environment was dissected and observed hourly under a dissection microscope starting on the fourth day following infestation. The time at which Hessian fly larvae were first seen at the base of examined plants was defined as the time for the initial attack.

Heat Stress Application. Wheat seedlings of Molly and Newton were transferred from a growth chamber under normal growth condition (20°C) to a growth chamber preadjusted to 40°C for 6 h. Heat stress was started at the same time for infested and noninfested plants at the time the initial attack took place in the infested plants. Wheat seedlings that were not subject to heat stress remained in the same growth chamber at 20°C.

Sample Collection. All samples were collected immediately at the time heat stress was completed. A 10-mm-long section of the second leaf sheath, where larvae resided if infested, was harvested for sampling. To inactivate lipases, samples were immediately transferred into 3 ml of isopropanol with 0.01% butylated hydroxytoluene preheated at 75°C in a 50-ml (25 by 150 mm) glass tube with a Teflon-lined screw cap for 15 min, and then 1.5 ml of chloroform and 0.6 ml of water were added to each of the samples. The samples were then stored at −80°C until extraction.

Lipid Extraction. Lipids were extracted from the samples using the method described by Welti et al. (2002). Briefly, samples in 1.5 ml of chloroform and 0.6 ml of water in the −80°C freezer were thawed and shaken for 1 h at room temperature. Lipid extracts for each sample were transferred to a glass tube with Teflon-lined screw-cap. Four milliliter of chloroform/methanol (2:1 in volume) with 0.01% butylated hydroxytoluene was added to plant tissues, which was shaken for 30 min to re-extract the lipids. The re-extractions were performed five times until plant tissues appeared white, and the extractions from each time were combined for the same sample. The extracts were washed once with 1 ml of 1 M KCl and once with 2 ml of water. After washing, the solvent was evaporated from the extracts with a nitrogen stream, and then the extracts were dissolved in 1 ml of chloroform. The remaining plant tissues were heated overnight at 105°C and weighed. The weight of the dried and ex-

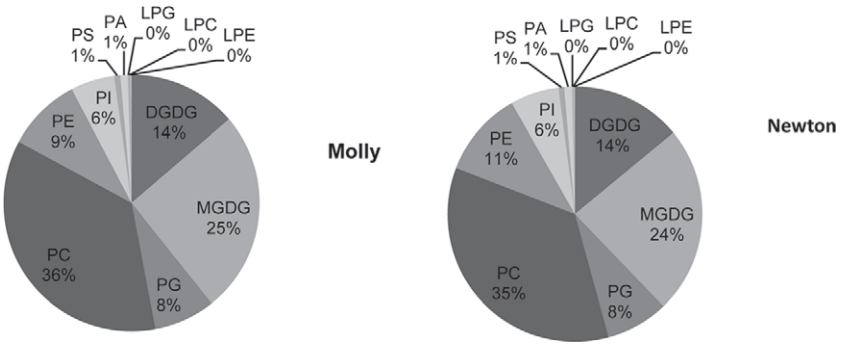

Fig. 1. Mole percentage of polar lipid classes in the control plants of Molly and Newton. The mole percentage of LPG, LPC, and LPE are 0.2, 0.1, and 0.2%, respectively, in Molly, and 0.1, 0.1, and 0.2%, respectively, in Newton. These numbers became 0% because of rounding down.

tracted tissue (dry weight minus lipids) is referred to as the "dry weight" of each sample.

Electrospray Ionization Tandem Mass Spectrometry Analysis. To analyze lipid profile, an automated electrospray ionization tandem mass spectrometry (ESI-MS/MS) approach was used and data acquisition was carried out as described by Xiao et al. (2010; supplemental data) with minor modifications, which are indicated in the following. The samples were dissolved in 1 ml of chloroform. An aliquot of 20 or 30 μl of extract in chloroform was used. Precise amounts of internal standards, obtained and quantified as previously described (Welti et al. 2002), were added in the following quantities: 0.60 nmol di14:0-PC; 0.60 nmol di24:1-PC; 0.60 nmol 13:0-lysoPC; 0.60 nmol 19:0-lysoPC; 0.30 nmol di14:0-PE; 0.30 nmol di24:1-PE; 0.30 nmol 14:0-lysoPE; 0.30 nmol 18:0-lysoPE; 0.30 nmol di14:0-PG; 0.30 nmol di24:1-PG; 0.30 nmol 14:0-lysoPG; 0.30 nmol 18:0-lysoPG; 0.30 nmol di14:0-PA; 0.30 nmol di20:0 (phytanoyl)-PA; 0.20 nmol di14:0-PS; 0.20 nmol di20:0 (phytanoyl)-PS; 0.23 nmol 16:0–18:0-PI; 0.16 nmol di18:0-PI; 2.01 nmol 16:0–18:0-MGDG; 0.39 nmol di18:0-MGDG; 0.49 nmol 16:0–18:0-DGDG; and 0.71 nmol di18:0-DGDG. The sample and internal standard mixture were combined with solvents, such that the ratio of chloroform/methanol/300 mM ammonium acetate in water was 300/665/35 in volume, and the final volume was 1.2 ml. The remainder of the procedure, i.e., data collection and processing, was conducted as described by Xiao et al. (2010). This procedure provides quantitative data in which lipids are determined in relation to internal standards of the same class. The values were reported as normalized mass spectral intensity in which a unit of 1 = the amount of normalized mass spectral intensity of 1 nmol of the corresponding internal standards. For phospholipids, the data correspond to the stated amounts of lipid (units: nmol lipid/mg dry weight) because analytes and internal standards have similar response factors. For galactolipids, there is some variability in responses among molecular species, and molecular species-specific response factors have not been applied. This does not compromise sample-to-sample comparisons. Furthermore, mole percentage of each lipid molecular species (lipid species), which refers to the percentage of total lipid mass spectral intensity contributed by a particular lipid molecular species, was calculated as the percent of the amount of lipid for each lipid species to the total amount of all lipid species.

Calculation of Double Bond Index (DBI). DBI was calculated for lipids in each head group class (lipid class) using the following equation: DBI_{class} = sum of $[N \cdot \text{mol}\%]$, where N is the number of double bonds in a lipid species (Chen et al. 2006, Su et al. 2009).

Statistical Analysis. Analysis of variance (ANOVA) was performed using general linear models procedure (SAS Institute 1999, Cary, NC). Means were compared, and the significance of difference was determined by Fisher's least significant difference (LSD, $\alpha = 0.01$). In addition, principal component analysis (PCA) was performed on mole percentage of samples from all treatments using the FactoMineR package (Husson et al. 2013) of the R software environment for statistical computing (R Core Team 2013, Vienna, Austria).

Results

Lipid Classes and Abundance in Leaf Sheath Tissue at the Hessian Fly Attack Site. One hundred and fifty-six lipid species were identified in the second leaf sheath of Molly and Newton. These lipid species belong to 11 polar lipid head group classes (lipid classes): digalactosyldiacylglycerol (DGDG, 16 species), monogalactosyldiacylglycerol (MGDG, 16 species), phosphatidylglycerol (PG, 13 species), phosphatidylcholine (PC, 20 species), phosphatidylethanolamine (PE, 23 species), phosphatidylinositol (PI, 14 species), phosphatidylserine (PS, 26 species), phosphatidic acid (PA, 12 species), lysosphatidylglycerol (LPG, 5 species), lysophosphatidylcholine (LPC, 6 species), and lysophosphatidylethanolamine (LPE, 5 species). In the control plants of Molly and Newton, in which heat stress and infestation were not applied, PC, MGDG, and DGDG were abundant lipid classes (Fig. 1), which together represented >70% of the detected polar lipids. PE, PG, PI, PA, and PS were less abundant, contributing from ≈1 to 10% of the amount of total detected lipids (total lipid). LPC, LPE, and LPG were

Table 2. Means ± SD of the amount (nmol/mg dry weight) of total lipid and each lipid classes in wheat seedlings of different treatment

	Control	Heat stress	Fly infestation	Combination treatment
Molly				
Total lipid	141.4 ± 4.29a	**125.8 ± 1.25b**	**114.7 ± 7.34bc**	**106.3 ± 8.66c**
DGDG	19.4 ± 0.93a	**17.1 ± 2.07ab**	**14.3 ± 1.26b**	**14.0 ± 2.12b**
MGDG	36.8 ± 3.32a	**33.3 ± 5.38ab**	**25.3 ± 0.91c**	**28.0 ± 2.95bc**
PG	11.0 ± 2.11a	**6.8 ± 1.25b**	12.2 ± 1.86a	**6.7 ± 00.78b**
PC	50.8 ± 7.60a	**44.5 ± 1.62ab**	**42.6 ± 4.47ab**	**36.2 ± 3.00b**
PE	13.2 ± 0.98a	**11.7 ± 0.83ab**	**11.4 ± 1.48b**	**10.2 ± 0.85b**
PI	7.9 ± 1.00ab	**9.6 ± 0.31a**	**6.2 ± 00.43b**	**8.2 ± 1.34a**
PS	1.1 ± 0.30ab	1.1 ± 0.13ab	**0.9 ± 0.08b**	**1.3 ± 0.13a**
PA	1.3 ± 0.37a	1.2 ± 0.17a	1.2 ± 0.34a	1.2 ± 0.53a
LPC	0.19 ± 0.05a	0.13 ± 0.010a	**0.11 ± 0.013b**	**0.12 ± 0.040ab**
LPE	0.25 ± 0.032a	**0.18 ± 0.018b**	**0.20 ± 0.026ab**	**0.15 ± 0.028b**
LPG	0.26 ± 0.13a	0.11 ± 0.096a	0.31 ± 0.10a	0.30 ± 0.127a
Newton				
Total lipid	134.7 ± 9.82a	**127.9 ± 6.88ab**	**117.9 ± 5.23bc**	**111.8 ± 4.84c**
DGDG	18.9 ± 1.46a	16.6 ± 1.30a	**13.7 ± 1.17b**	**13.1 ± 1.12b**
MGDG	32.0 ± 1.87a	33.9 ± 3.49a	**22.9 ± 1.84b**	**21.8 ± 0.96b**
PG	10.7 ± 00.67a	**6.9 ± 0.63b**	10.6 ± 1.36a	**6.3 ± 0.04b**
PC	47.4 ± 4.91a	45.5 ± 2.08a	47.6 ± 4.60a	46.6 ± 4.06a
PE	14.7 ± 1.06a	**12.1 ± 0.26b**	**13.1 ± 1.32b**	**12.3 ± 0.82b**
PI	8.3 ± 0.65bc	**10.2 ± 0.82a**	**7.7 ± 0.35c**	**9.3 ± 0.39ab**
PS	1.0 ± 0.14b	**1.1 ± 0.02a**	**0.7 ± 0.10c**	**1.0 ± 0.06b**
PA	10.3 ± 0.22a	1.1 ± 1.07a	1.2 ± 0.18a	1.0 ± 0.27a
LPC	0.14 ± 0.023a	0.13 ± 0.005a	0.13 ± 0.015a	0.13 ± 0.02a
LPE	0.24 ± 0.019a	**0.18 ± 0.010b**	**0.19 ± 0.018b**	**0.17 ± 0.02b**
LPG	0.12 ± 0.054a	0.09 ± 0.049a	0.11 ± 0.045a	0.05 ± 0.02a

Means in the same row marked with different letters are significantly different using the Fisher's LSD multiple comparison test at $\alpha = 0.01$. Values that are statistically different from the control plants were bold faced. $N = 5$.

Table 3. Means ± SD of DBI of major lipid classes in different treatment

	Control	Heat stress	Fly infestation	Combination treatment
Molly				
DGDG	4.7 ± 0.03b	4.6 ± 0.06b	**4.8 ± 0.05a**	**4.8 ± 0.05a**
MGDG	5.5[a] ± 0.04b	**5.3 ± 0.03d**	**5.7 ± 0.02a**	**5.5[b] ± 0.03c**
PG	2.8 ± 0.04a	**2.4 ± 0.04b**	2.7 ± 0.05a	**2.4 ± 0.06b**
PC	3.7 ± 0.16a	3.6 ± 0.02a	3.7 ± 0.04a	3.7 ± 0.05a
PE	4.2 ± 0.05a	**3.9 ± 0.08b**	4.1 ± 0.03a	**3.9 ± 0.06b**
PI	3.1 ± 0.02a	**2.9 ± 0.01b**	3.0 ± 0.02a	**2.9 ± 0.03b**
PS	3.2 ± 0.04a	**3.0 ± 0.04b**	**3.0 ± 0.05b**	**2.9 ± 0.11c**
PA	3.4 ± 0.05a	3.3 ± 0.10a	3.4 ± 0.05a	3.4 ± 0.09a
Newton				
DGDG	4.7 ± 0.06a	4.6 ± 0.03a	4.7 ± 0.07a	4.7 ± 0.07a
MGDG	5.6 ± 0.03a	**5.4 ± 0.03b**	5.6 ± 0.03a	5.6 ± 0.04a
PG	2.9 ± 0.05a	**2.5 ± 0.03d**	**2.8 ± 0.05b**	**2.6 ± 0.05c**
PC	4.0 ± 0.09a	4.0 ± 0.04a	3.9 ± 0.09a	4.0 ± 0.09a
PE	4.2 ± 0.06a	**4.0 ± 0.04b**	4.2 ± 0.06a	**4.0 ± 0.03b**
PI	3.1[c] ± 0.02a	**2.9 ± 0.02d**	**3.1[d] ± 0.02b**	**3.0 ± 0.01c**
PS	3.3 ± 0.02a	**3.1 ± 0.04c**	**3.2 ± 0.02b**	**3.1 ± 0.02c**
PA	3.6 ± 0.07a	**3.5 ± 0.09ab**	**3.4 ± 0.07b**	**3.4 ± 0.02b**

Fisher's LSD test was conducted to compare means among treatments. Means in the same row marked with different letters are significantly different at $\alpha = 0.01$. Values that are statistically different from the control plants are bold faced. $N = 5$.

[a] Rounded from 5.54.
[b] Rounded from 5.45.
[c] Rounded from 3.13.
[d] Rounded from 3.06.

the least abundant, which together constituted <1%. The composition (mol%) of each lipid class was similar between Molly and Newton (Fig. 1).

Reductions in Lipid Abundance Induced by Heat Stress, Hessian Fly Infestation, or the Combination Treatment. In both Molly and Newton, heat stress, Hessian fly infestation (fly infestation), and the combination of heat stress and fly infestation (combination treatment) caused reductions in the abundance of total lipid (Table 2), and such an effect was in the following order: Heat stress < fly infestation < combination treatment. Specifically the amount of total lipid was reduced by 11.0, 18.9, and 24.8%, respectively, in Molly, and by 5.0, 12, and 17%, respectively, in Newton following heat stress, fly infestation, and the combination treatment.

The abundance of all lipid classes except PA and LPG was affected by at least one of these stress conditions in Molly or Newton (Table 2). Among the nine affected lipid classes, the effect of heat stress and fly infestation exerted similar effect on the abundance of lipid classes DGDG, PE, and LPE in both lines. Specifically, both stress conditions reduced or tended to reduce the abundance (nmol lipid/mg dry weight) of these lipid classes in Molly and Newton. The effect of heat stress and fly infestation was different on the abundance of classes PG, PI, and PS. Specifically, in both lines, the abundance of class PG decreased after heat stress but was not affected by fly infestation; the abundance of class PI increased after heat stress, but decreased after fly infestation; the abundance of class PS decreased after fly infestation, but either increased or not affected under heat stress (Table 2). In addition, the effect on the abundance of class PC was different between Molly and Newton. In particular, the abundance of class PC decreased after heat stress, fly infestation, and the combination treatment in Molly, but was not affected in Newton. Together these results indicated that significant differences in lipid abundance were induced under different stress conditions and between these NILs.

Changes in DBI After Different Treatments. DBIs represent the unsaturation levels of lipids. A greater DBI indicates a more unsaturation in membrane lipids (Alam and Alam 1986, Chen et al. 2006, Su et al. 2009). Heat stress caused significant reduction in DBIs in five lipid classes including MGDG, PG, PE, PI, and PS in both Molly and Newton, indicating that heat stress induced decreases in unsaturation levels of polar lipids, and that the effect of heat stress was similar between Molly and Newton (Table 3). Fly infestation, however, affected the DBIs of these lipid classes in Molly and Newton differently. In Molly, fly infestation induced increases in the DBIs of DGDG and MGDG, but a decrease in the DBI of PS. In Newton, however, fly infestation caused decreases in DBIs of PG, PI, PS, and PA. The combination treatment caused an increase in the DBI of DGDG but decreases in the DBIs of MGDG, PG, PE, PI, and PS in Molly, and decreases in the DBIs of PG, PE, PI, PS, and PA in Newton (Table 3).

Changes in the Compositions of Lipid Species Caused by Stress Conditions. The lipid composition is referred to as the mole percentage of each lipid species, which is indicative of the relative abundance of each lipid species. Of the 156 lipid species detected, the composition of 98 (63%) across all 11 lipid classes was significantly affected by at least one of the stress conditions in Molly and/or Newton (Table 4). Heat

Table 4. Lipid species affected by heat stress, fly infestation, or the combination treatment in Molly and Newton

Lipid Species[a]	Heat stress		Fly infestation		Combination treatment	
	Molly	Newton	Molly	Newton	Molly	Newton
DGDG (34:3)				−19.8		−17.4
DGDG (34:2)	**49.5**	**30.1**				
DGDG (34:1)			−30.1		−26.6	
DGDG (36:5)		16.5				−17.1
DGDG (36:4)	**−23.5**	**−23.0**	**−31.1**	**−16.5**	**−40.3**	**−32.9**
DGDG (36:3)			−29.4		**−34.1**	**−35.6**
DGDG (36:2)		−23.0	−43.7		**−56.6**	**−32.5**
DGDG (38:6)				32.0	**66.5**	**47.4**
DGDG (38:4)	−26.2		−32.8		**−46.9**	**−27.5**
DGDG (38:3)			−83.7		**−64.9**	**−53.0**
MGDG (34:5)					456.1	
MGDG (34:4)				−35.3		−37.5
MGDG (34:3)			**−35.9**	**−30.6**		−28.9
MGDG (34:2)	**147.0**	**92.5**				
MGDG (34:1)		−26.7	−54.0		**−58.0**	**−66.4**
MGDG (36:6)	**−21.0**	**−14.7**		−19.4		−21.0
MGDG (36:5)	**75.8**	**121.0**			46.1	
MGDG (36:4)	**58.3**	**86.5**	−47.3			
MGDG (36:3)				−21.0	16.2	−26.1
MGDG (36:2)			−42.4		**−48.3**	**−72.5**
MGDG (38:5)					626.6	
MGDG (38:4)						−79.2
PG (32:0)	**77.3**	**69.3**	62.7		**181.2**	**115.7**
PG (34:3)	**−48.2**	**−49.5**	31.9		**−40.3**	**−39.9**
PG (34:2)		28.1	**62.3**	**37.3**		
PG (34:1)				27.0		−25.8
PG (36:6)	**−53.2**	**−55.9**		−14.6	**−43.2**	**−45.4**
PG (36:5)			**52.2**	**26.8**		
PG (36:4)		−39.2	37.0		**−34.0**	**−43.7**
PG (36:3)			**106.3**	**82.3**		
PG (36:2)						−41.0
LPC (16:0)					98.9	
LPC (16:1)	−76.4		−95.1	226.5	−91.6	
LPC (16:0)	−34.4			61.4	−35.0	
LPC (18:1)				81.5		
LPC (18:0)			−88.2		−77.4	
LPE (16:1)	−83.3				−100.0	
LPE (16:0)					−24.2	
LPE (18:3)	**−36.1**	**−30.8**			**−45.0**	**−27.9**
LPE (18:1)						−95.9
PC (32:0)			−32.0			202.3
PC (34:4)						31.0
PC (34:3)		−11.6				
PC (34:2)		23.2		51.6		35.8
PC (34:1)			−38.0		−38.6	
PC (36:6)				−19.0		18.6
PC (36:4)				30.1		35.7
PC (36:3)	47.1					
PC (38:6)	35.4					77.3
PC (38:5)	33.0				**48.4**	**70.9**
PC (38:4)	−21.3				−25.7	
PC (38:3)				63.0		
PC (38:2)			−43.2		−56.2	
PE (32:2)	**113.9**	**86.0**			**116.3**	**99.6**
PE (32:1)		63.2			39.0	
PE (34:3)		−25.6				−15.3
PE (34:2)	**89.0**	**47.1**			**95.6**	**63.8**
PE (34:1)			−26.31			
PE (36:6)	**−42.1**	**−44.0**	−13.24		**−41.2**	**−32.9**
PE (36:5)						30.5
PE (36:4)	38.6		24.95		**38.1**	**33.6**
PE (36:1)				129.5		
PE (38:6)		−25.5				
PE (38:5)			38.66		51.3	
PE (38:4)	**−33.6**	**−49.3**			**−35.5**	**−42.3**
PE (40:2)		58.2	72.53		78.8	
PE (42:4)	**−52.6**	**−47.0**			**−42.7**	**−37.3**
PE (42:2)	**93.7**	**100.4**	88.17		**103.5**	**101.0**
PI (32:1)	**94.5**	**83.5**			**67.5**	**74.9**
PI (32:0)	**115.0**	**148.5**			**73.5**	**181.4**
PI (34:3)	**35.5**	**28.6**			**37.1**	**30.3**
PI (34:2)	**91.5**	**82.6**		25.1	**102.5**	**87.8**
PI (34:1)			−29.80	39.7		
PI (36:6)		−29.7				
PI (36:5)				17.9		14.1
PI (36:4)				17.9		
PI (36:3)				56.6		
PI (36:2)	57.4			42.5		
PS (34:4)						−87.0
PS (34:2)	**133.8**	**162.1**			**142.0**	**202.2**
PS (34:1)			213.07		205.4	
PS (36:6)	**−47.5**	**−52.8**		−37.5	**−53.4**	**−52.2**
PS (36:4)		−29.0		−27.2		−32.4
PS (36:3)	110.1		124.71		226.6	
PS (36:2)		63.0	664.26		**960.7**	**110.2**
PS (38:5)				−38.2	307.5	
PS (38:4)		−30.7				−29.4
PS (38:3)	42.4				38.2	
PS (38:2)		121.8				106.0
PS (40:3)	**37.2**	**31.5**				33.4
PS (40:2)	**88.3**	**91.9**			**110.0**	**70.4**
PS (40:1)				373.3		376.2
PS (42:3)		114.0			**126.4**	**71.9**
PS (42:2)			1,213.56		2,206.3	
PS (42:1)			678.64			
PA (34:1)		−29.1				
PA (36:6)						−38.2
PA (36:3)				61.9		

Numbers are percentage changes to the values of control plants at $\alpha = 0.01$. The percentage change was calculated by (treatment–CK)/CK × 100. Lipids species affected in the same direction between Molly and Newton by the same stress condition were bold faced. Blank cells indicate that the change was not statistically different compared with the control plants at $\alpha = 0.01$.

[a] The number before ":" represents the number of carbon in the side chain of each lipid species, and the number after ":" represents the number of double bonds.

stress significantly affected the composition of 54 lipid species, among 24 (44%) were changed in the same direction (Table 4), meaning that the composition of these species was either increased or decreased in both wheat lines. Infestation affected the compositions of 57 lipid species, of which five (9%) were changed in the same direction in both Molly and Newton (Table 4). Together, the above results indicated that changes in lipid composition induced after the heat stress were similar between Molly and Newton, but changes induced by infestation were distinctively different between these wheat lines. The combination treatment affected compositions of 77 species, 30 (39%) of them changed in the same directions between Molly and Newton. Of all lipid species, 34:3- and 36:4-PG were reduced in both Molly and Newton by the combination treatment, but increased in Molly after fly infestation (Table 4).

Changes in Lipid Profiles Under Different Stress Conditions. To provide an overall evaluation of the impact of these stress conditions on lipid profiles, PCA was conducted on the compositions of the 156 detected lipid species. The first two components, which explained 36% of the variance, were used to construct the score plot (Fig. 2). In the score plot, eight treatments formed four distinctive groups. The controls of Molly and Newton clustered together with the infested Newton, demonstrating that Molly and Newton has similar lipid profiles, and that infestation does not

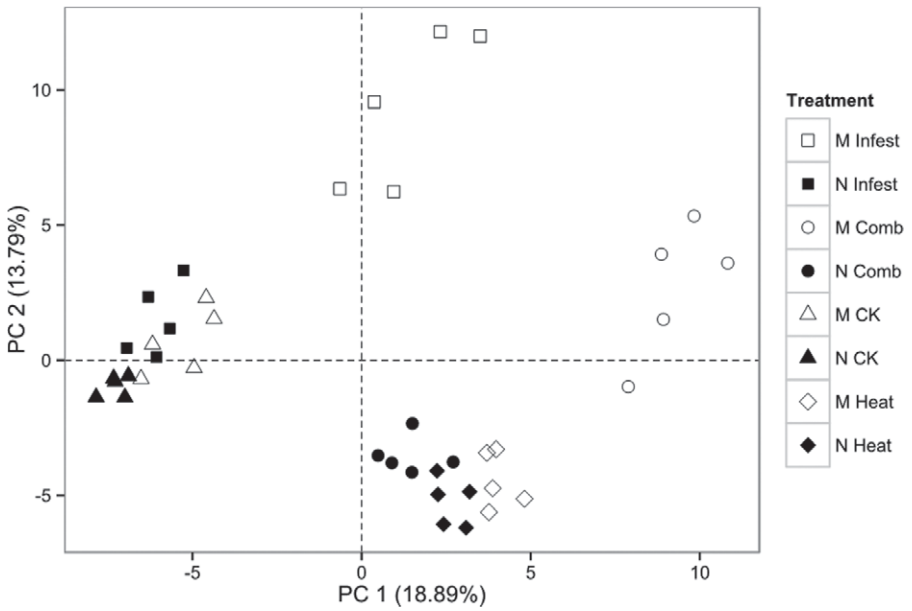

Fig. 2. Plot derived from the PCA on mole percentage of 156 lipid species of wheat seedlings in various treatment. M CK, Molly control; N CK, Newton control; M Heat, Molly subjected to the heat stress, N Heat, Newton subjected to the heat stress; M Infest, Molly infested with Hessian fly; N Infest, Newton infested with Hessian fly; M Comb, Molly subjected to the combination treatment, N Comb, Newton subjected to the combination treatment.

significantly affect lipid profiles in Newton. Heat-stressed Molly and Newton clustered with Newton under the combination treatment, indicating that effect of heat stress on lipid profiles of Molly and Newton, and the effect of the combination treatment on Newton were similar. The fly-infested Molly plants and Molly plants under the combination treatment each formed a unique group, indicating that fly infestation and the combination treatment affect lipid profiles in Molly plants in different ways.

Discussion

Differential Impacts of Heat Stress and Fly Infestation. Because Molly and Newton are NILs differing largely only in their reactions to Hessian fly infestation, expectedly, the control plants of Molly and Newton exhibited similar lipid profiles (Figs. 1 and 2). Heat stress alone imposed similar impacts on lipid profiles in Molly and Newton (Fig. 2), suggesting that these two lines adopt similar mechanisms to adapt to heat stress. In contrast, fly infestation caused distinctive changes in lipid profiles between Molly and Newton (Fig. 2 and Table 4). In Molly, the changes due to infestation are substantial and unique, and thus the fly-infested Molly sample formed a distinctive group in the PCA plot (Fig. 2). In Newton, however, the change in lipid profile caused by fly infestation was subtle, and as a result, the fly-infested Newton and control Newton clustered together in the PCA plot (Fig. 2). The significant difference in the fly infestation-induced changes between Molly and New reflects, at least to a certain degree, the different nature of reactions between Molly and Newton to fly infestation, i.e., the incompatibility of Molly versus the compatibility of Newton to the Hessian fly population used in this study. Compared with Newton, the dramatic changes of lipid profiles after only 6 h following fly infestation in Molly suggests that Molly responds to fly infestation more fiercely and more rapidly in lipid metabolism than its susceptible counterpart Newton. Because membrane lipids constitute the first layer of plant defenses and directly contribute to the synthesis of defense signals such as JA and OPDA (Kachroo and Kachroo 2009, Kosma et al. 2010), the more rapid and fierce responses concerning lipid metabolism in Molly may lead to a more rapid mobilization of a greater amount of resources to defend against Hessian fly than that in Newton, resulting in resistance in Molly to fly infestation. Such result is consistent with a previous study (Zhu et al. 2012). The weaker or slower responses in lipid metabolism in Newton to fly infestation may be part of the reason why Newton was unable to amount effective defense against the insect, leading to its susceptibility to Hessian fly infestation.

The difference between the impact of heat stress and fly infestation on lipid metabolism was also revealed by their impacts on DBIs, which reflect the unsaturation levels of membrane lipids (Chen et al. 2006, Su et al. 2009). Our study has indicated that the heat stress, though for a short period of time, decreased the unsaturation level of membrane lipids significantly in both Molly and Newton, which was indicated by the reduced DBIs of five lipid classes including MGDG, PG, PE, PI, and PS. In contrast, the fly infestation affected DBI differently between Molly

and Newton. For example, DBIs of DGDG and MGDG increased by fly infestation in Molly but such an effect was not observed in Newton, and the DBI of PG and PI decreased by fly infestation in Newton but not in Molly (Table 3). Interestingly, the increase of DBIs only occurred in the fly-infested Molly and Molly under the combination treatment, and all other stress conditions in Molly or Newton only caused reductions in DBIs in lipid classes. Such results indicated that decreased lipid unsaturation in membrane lipids may help plants adapt and survive under high temperature as demonstrated in a previous study (Iba 2002). Given that the fly-infested Newton is susceptible irrespective of heat stress, and that Molly treated with the combination of heat stress and fly infestation is largely susceptible to the otherwise avirulent Hessian fly population, our results indicated that the reduced lipid unsaturation in membrane lipids may be related to the susceptibility of plants to Hessian fly infestation.

Both heat stress and fly infestation caused a reduction in the abundance of total lipids (Table 2). However, these two stress conditions affected MGDG, DGDG, and PG in different ways. In both lines, heat stress caused a significant decrease in the abundance of class PG but its effects on that of classes MDGD and DGDG were hardly perceived; however, fly infestation caused a decrease in the abundance of classes MGDG and DGDG, but it did not affect that of PG. These three lipid classes are critical component of plastid membrane and their abundances directly affect functionality of photosynthesis (Hagio et al. 2002, Sakurai et al. 2007). The differential impacts on MGDG, DGDG, and PG by heat stress and fly infestation seemed to suggest that both heat stress and fly infestation affected the photosynthesis of plants, but through different pathways and mechanisms.

Heat-Induced Loss of Hessian Fly Resistance and Lipid Metabolism in Wheat Seedlings. In a previous study, we have found that under heat stress of 40°C for 6 h, 50–80% of Molly plants became susceptible and allowed avirulent larvae to survive and develop in these otherwise resistant plants (Currie et al. 2014). The results of our current study seemed to suggest that lipid metabolic pathways could be one of the mechanisms through which the resistance of Molly was suppressed by the heat stress (Fig. 2). Despite distinct differences in lipid profiles of the fly-infested Molly and Newton that correspond to their distinct reactions to avirulent Hessian fly, when infested and subjected to heat stress, Molly produced lipid profiles different from the infested Molly in the incompatible interaction (Fig. 2). These results suggest that the heat-induced loss of resistance may be related to the changed lipid metabolism of Molly under heat stress. Our results in DBIs further supported such a possibility (Table 3). Without heat stress, DBIs between Molly in the incompatible interaction and Newton in the compatible interaction were distinctively different. However, in heat-stressed and fly-infested plants, DBIs between Molly and Newton became much similar, which was also similar to that of Newton infested with Hessian fly alone (Table 3). The similarity in DBIs between Newton in the compatible interaction and the largely susceptible Molly owing to the heat stress further suggests that the heat stress-induced changes in lipid metabolism may be related to the heat-induced susceptibility in Molly. The heat-induced susceptibility in Molly is likely associated with the decreased accumulation of OPDA caused by heat stress, as OPDA is an necessary defense signaling molecule in the resistance of wheat to fly infestation (Zhu et al. 2010) and its synthesis is directly linked to lipid metabolism (Turner et al. 2002).

In conclusion, our study suggests that the transient heat stress caused significant reductions in the abundance of membrane lipids and their unsaturation levels. Wheat plants respond to heat stress and fly infestation differently in lipid metabolism. Heat stress on infested Molly plants generates unique changes in lipid metabolisms. Heat stress-induced changes in lipid metabolism may be attributable to the loss of resistance in Molly plants to the otherwise avirulent Hessian fly population.

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References Cited

Alam, S. Q., and B. S. Alam. 1986. Effect of essential fatty acid deficiency on acyl group composition, membrane fluidity and adenylate cyclase activity in plasma membranes of rat submandibular salivary glands. J. Nutr. 116: 1620–1630.

Berzonsky, W. A., H. Ding, S. D. Haley, M. O. Harris, R. J. Lamb, R.I.H. McKenzie, H. W. Ohm, F. L. Patterson, F. B. Peairs, D. R. Porter, et al. 2003. Breeding wheat for resistance to insects. Plant Breed. Rev. 22: 221–296.

Buntin, G. D., P. L. Bruckner, J. W. Johnson, and J. E. Foster. 1990. Effectiveness of selected genes for Hessian fly resistance in wheat. J. Agric. Entomol. 7: 283–291.

Buntin, G. D., S. L. Ott, and J. W. Johnson. 1992. Integration of plant resistance, insecticides, and planting date for management of the Hessian fly (Diptera: Cecidomyiidae) in winter wheat. J. Econ. Entomol. 85: 530–538.

Chen, J., J. J. Burke, Z. Xin, C. Xu, and J. Velten. 2006. Characterization of the Arabidopsis thermosensitive mutant atts02 reveals an important role for galactolipids in thermotolerance. Plant Cell Environ. 29: 1437–1448.

Currie, Y., J. Moch, J. Underwood, H. Kharabsheh, A. Quesenberry, R. Miyagi, C. Thomas, M. Boney, S. Wood, M. S. Chen, et al. 2014. Transient heat stress compromises the resistance of wheat seedlings to Hessian fly (Diptera: Cecidomyiidae) infestation. J. Econ. Entomol. 107: 389–395.

Garcés-Carrera, S., A. Knutson, H. Wang, K. L. Giles, F. Huang, R. J. Whitworth, C. M. Smith, and M. Chen. 2014. Virulence and biotype analyses of Hessian fly (Diptera: Cecidomyiidae) populations from Texas, Louisiana, and Oklahoma. J. Econ. Entomol. 107: 417–423.

Garrett, K. A., S. P. Dendy, E. E. Frank, M. N. Rouse, and S. E. Travers. 2006. Climate change effects on plant disease: genomes to ecosystems. Annu. Rev. Phytopathol. 44: 489–509.

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

Gould, F. 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. Annul. Rev. Entomol. 43: 701–726.

Hagio, M., I. Sakurai, S. Sato, T. Kato, S. Tabata, and H. Wada. 2002. Phosphatidylglycerol is essential for the development of thylakoid membranes in *Arabidopsis thaliana.* Plant Cell Physiol. 43: 1456–1464.

Hatchett, J. H., and R. L. Gallun. 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.

Husson, F., J. Josse, S. Le, and J. Mazet. 2013. FactoMineR: Multivariate exploratory data analysis and data mining with R. R package version 1.25. (http://CRAN.R-project.org/package=FactoMineR).

Iba, K. 2002. Acclimative response to temperature stress in higher plants: approaches of gene engineering for temperature tolerance. Annu. Rev. Plant Biol. 53: 225–245.

Isaak, A., E. L. Sorensen, and E. E. Ortman. 1963. Influence of temperature and humidity on resistance in alfalfa to the spotted alfalfa aphid and pea aphid. J. Econ. Entomol. 56: 53–57.

Kachroo, A., and P. Kachroo. 2009. Fatty acid-derived signals in plant defense. Annu. Rev. Phytopathol. 47: 153–176.

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

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

Liu, X., C. Khajuria, J. Li, H. N. Trick, L. Huang, B. S. Gill, G. R. Reeck, G. Antony, F. F. White, and M. Chen. 2013. Wheat Mds-1 encodes a heat-shock protein and governs susceptibility towards the Hessian fly gall midge. Nat. Comm. 4: 2070 doi:10.1038/ncomms3070.

McMahon, H. T., and J. L. Gallop. 2005. Membrane curvature and mechanisms of dynamic cell membrane remodeling. Nature 438: 590–596.

Patterson, F. L., F. B. Maas, J. E. Foster, R. H. Ratcliffe, S. Cambron, G. Safranski, P. L. Taylor, and H. W. Ohm. 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.

Ratcliffe, R. H., S. E. Cambron, K. L. Flanders, N. A. Bosque-Perez, S. L. Clement, and H. W. Ohm. 2000. Biotype composition of Hessian fly (Diptera: Cecidomyiidae) populations from the southeastern, midwestern, and northwestern United States and virulence to resistance genes in wheat. J. Econ. Entomol. 93: 1319–1328.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (http://www.R-project.org/).

Sakurai, I., N. Mizusawa, S. Ohashi, M. Kobayashi, and H. Wada. 2007. Effects of the lack of phosphatidylglycerol on the donor side of photosystem II. Plant Physiol. 144: 1336–1346.

SAS Institute. 1999. SAS/STAT user's guide, version 6. SAS Institute. Cary, NC.

Shukle, R. H., and J. J. Stuart. 1993. A novel morphological mutation in the Hessian fly, *Mayetiola destructor.* J. Hered. 84: 229–232.

Stuart, J. J., M. S. Chen, R. Shukle, and M. O. Harris. 2012. Gall midges (Hessian Flies) as plant pathogens. Annu. Rev. Phytopathol. 50: 339–357.

Su, K., D. J. Bremer, R. Jeannotte, and R. Welti. 2009. Membrane lipid composition and heat tolerance in cool-season turfgrasses including a hybrid bluegrass. J. Am. Soc. Hort. 134: 511–520.

Thindwa, H. P., and G. L. Teetes. 1994. Effect of temperature and photoperiod on sorghum resistance to biotype C and E greenbug (Homoptera: Aphididae). J. Econ. Entomol. 87: 1366–1372.

Turner, J. G., C. Ellis, and A. Devoto. 2002. The jasmonate signal pathway. Plant Cell 14: 153–164.

Tyler, J. M., and J. H. Hatchett. 1983. Temperature influence on expression of resistance to Hessian fly (Diptera: Cecidomyiidae) in wheat derived from *Triticum tauschii.* J. Econ. Entomol. 76: 323–326.

Van Meer, G., D. R. Voelker, and G. W. Feigenson. 2008. Membrane lipids: where they are and how they behave. Nat. Rev. Mol. Cell Biol. 9: 112–124.

Wang, Y., Z. Bao, Y. Zhu, and J. Hua. 2009. Analysis of temperature modulation of plant defense against biotrophic microbes. Mol. Plant Microbe Interact 22: 498–506.

Welti, R., W. Li, M. Li, Y. Sang, H. Biesida, H. Zhou, C. B. Rajashekar, T. D. Williams, and X. Wang. 2002. Profiling membrane lipids in plant stress responses. J. Biol. Chem. 277: 31994–32002.

Whitham, S., S. McCormick, and B. Baker. 1996. The N gene of tobacco confers resistance to tobacco mosaic virus in transgenic tomato. Proc. Natl. Acad. Sci. USA 93: 8776–8781.

Xiao, S., W. Gao, Q. Chen, S. Chan, S. Zheng, J. Ma, M. Wang, R. Welti, and M. Chyea. 2010. Overexpression of *Arabidopsis* acyl-CoA binding protein ACBP3 promotes starvation induced and age-dependent leaf senescence. Plant Cell 22: 1–20.

Zhu, L., X. M. Liu, X. Liu, R. Jeannotte, J. C. Reese, R. Welti, M. Harris, J. J. Stuart, and M. S. Chen. 2008. Hessian fly (*Mayetiola destructor*) attack causes a dramatic shift in carbon and nitrogen metabolism in wheat. Mol. Plant Microbe Interact 21: 70–78.

Zhu, L., X. Liu, and M. S. Chen. 2010. Differential accumulation of phytohormones in wheat seedlings attacked by avirulent and virulent Hessian fly (Diptera: Cecidomyiidae) larvae. J. Econ. Entomol. 103: 178–185.

Zhu, L., X. Liu, H. Wang, C. Khajuria, J. C. Reese, R. J. Whitworth, and M. S. Chen. 2012. Rapid mobilization of membrane lipids in wheat leaf-sheaths during incompatible interactions with Hessian fly. Mol. Plant Microbe Interact 25: 920–930.

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