LIPID PROFILES IN WHEAT CULTIVARS RESISTANT AND SUSCEPTIBLE TO TAN SPOT AND THE EFFECT OF DISEASE ON THE PROFILES

by

DONG WON KIM

M.S., Konkuk University, 2006

A THESIS

Submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Plant Pathology
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2012

Approved by:

Major Professor
William W. Bockus

Abstract

The effects of tan spot on lipid profiles in wheat leaves were quantified by mass spectrometry. Inoculation with *Pyrenophora tritici-repentis* significantly reduced the amount of many lipids, including the major lipids monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), in leaves over time. These two lipids accounted for 89% of the mass spectral signal of detected lipids in wheat leaves. Reductions in amounts of lipids were at much higher rates over time for susceptible cultivars compared with resistant cultivars. Furthermore, data show that cultivars resistant to tan spot have different lipid profiles when compared with susceptible cultivars. Resistant cultivars had more MGDG and DGDG than susceptible ones, even in non-inoculated leaves. Using linear models that were fit to data, non-inoculated cultivars with a rating of 1 (highly resistant to tan spot) were calculated to have 66.1% more MGDG and 52.7% more DGDG signal than cultivars with a rating of 9 (highly susceptible). These latter findings are indirect evidence that the amounts of some lipids in wheat leaves may be determining factors in the resistance response of cultivars to tan spot.

Table of Contents

List of Figures........ iii

List of Tables v

Introduction........ 1

Materials and Methods........ 7

Results........ 10

Discussion........ 13

References........ 18

Figures........ 25

Tables........ 30

Appendices........ 34

Appendix 1: Profiles of major lipid molecular species........ 34

Appendix 2: Profiles of minor lipid molecular species........ 35

List of Figures

Figure 1. The distribution of detected lipid classes..25

Figure 2. Comparison of MGDG...26

Figure 3. Comparison of DGDG..27

Figure 4. Correlation of MGDG and the level of resistance to tan spot............................28

Figure 5. Correlation of DGDG and the level of resistance to tan spot.............................29

List of Tables

Table 1. Reaction of 20 selected wheat cultivars to tan spot..30

Table 2. Comparison of amount of lipid in healthy and tan spot-infected wheat leaves..31

Table 3. Comparison of amount of lipid between wheat cultivars resistant and susceptible to tan spot..32

Table 4. Comparison of slopes and estimated intercepts for amount of MGDG and DGDG regressed against harvest time for wheat cultivars resistant and susceptible to tan spot...33

Introduction

Wheat is an important source of calories for humans and animals. In the U.S., wheat is consumed by humans in various products such as bread, pasta, and pizza. Worldwide wheat production in 2009 was estimated at 25 billion bushels and 2.2 billion bushels were produced in the U.S. In Kansas, 360 million bushels of wheat were produced in 2010 (USDA, 2010) and that state has produced over 400 million bushels in 12 of the last 33 years. However, wheat is exposed to various abiotic or biotic stresses which reduce production. Drought, heat, and cold are abiotic stresses that commonly affect wheat production, while insect and disease threats are biotic agents that can lead to reduced yields. These limitations can affect both the quality and quantity of wheat.

Tan spot, which is caused by the fungus *Pyrenophora tritici-repentis* (Died.) Drechsler, is one of the major foliar diseases of wheat worldwide (De Wolf et al, 1998) and causes losses up to 50% (Shabeer and Bockus, 1988; Singh and Hughes, 2005). Increases in disease incidence have been attributed to changes in cultural practices (Lamari and Bernier, 1989) such as shifts from conventional tillage to conservation- and zero-tillage, shorter crop rotations, continuous wheat cultivation, and the culture of highly susceptible cultivars (Ciufetti et al., 1999). The fungus overwinters as fruiting bodies called pseudothecia that develop on the previous season's infected wheat residue on the soil surface. Pseudothecia release sexual spores (ascospores) in the spring that induce the first infections of the growing season. Asexual spores (conidia) are produced on crop residue and from leaf spots. Conidia are dispersed by wind and germinate to infect wheat in a wide range of temperatures but require continual leaf wetness for at least 6 hours (McMullen, 2010). During the growing season, many conidia form in the lesions and

serve as the secondary inoculum to produce the epidemic (McMullen and Adhikari, 2009).

Tan spot displays two main phenotypic symptoms on wheat leaves, necrosis and chlorosis. These symptoms are induced by at least three host-specific toxins which are designated Ptr ToxA, Ptr ToxB, and Ptr Tox C (Strelkov and Lamari, 2003). These toxins are important in the tan spot/wheat pathosystem because the eight races of the fungus are classified based on the putative production or nonproduction of these toxins. Toxin production is deduced because of their reaction on a set of differential wheat lines/cultivars (Lamari and Strelkov, 2010). Ptr Tox A is the best characterized toxin and was the first to be isolated (Ballance et al., 1989; Tomas et al., 1990; Tuori et al., 1995). It is responsible for the necrosis symptom on sensitive wheat genotypes and is a 13.2-kDa protein encoded by the *ToxA* gene (Ballance et al., 1996; Ciuffetti et al., 1997). Ptr Tox B is also a small (6.6 kDa) protein molecule and is encoded by the *ToxB* gene; it induces chlorosis on sensitive wheat genotypes (Strelkov et al., 1998). Ptr Tox C also induces chlorosis but on different wheat lines/cultivars (Gamba et al., 1998). It is not proteinaceous like Ptr Tox A and Ptr Tox B but is a non-ionic, polar, low-molecular mass molecule (Effertz et al., 2002).

Lipids are important molecules in living organisms but definitions of lipids are varied. The definition used here is that lipids are biomolecular compounds that are soluble in organic solvents such as chloroform, benzene, ethers, and alcohols (Buchanan et al., 2000; Voet et al., 2006). Another important aspect of lipids is found in Christie's (2011) definition; lipids are "fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds." Lipids are important to plants for

energy storage and the formation of protective surfaces on cells and plant leaves, stems, and roots (Graham et al., 2003). Also, they are important for photosynthesis and serve as messengers in signal transduction mechanisms that influence plant growth, development, and response to stress (Shah, 2005). Fatty acids are carboxylic acids with reduced long chain hydrocarbon side groups and most lipids contain fatty acids esterified to glycerol. Lipids containing hydrocarbon chains store energy. Fatty acids are substantially more reduced organic molecules than carbohydrates and oxidation of fatty acid has a higher potential for producing energy (Buchanan, 2000; Voet et al., 2006). Therefore, lipids with more fatty acids have advantages for production of energy. In their role of energy storage, plant lipids represent a highly reduced form of carbon and the simple form is triacylglycerol which contains three fatty acids. Energy storage lipids serve as a source of energy during seed germination and seedling development. Also, for humans, most of the plant storage lipids are consumed as edible oils (Buchanan et al., 2000; Weselake, 2005).

Membranes not only separate the interior of cell contents, such as chloroplasts and mitochondria, from the cytoplasm, but they also are selectively permeable to ions and organic molecules. This helps to control the movement of substances in and out of cells (Albert et al., 2002; Buchanan et al., 2000). Biological cell membranes consist of a lipid bilayer composed primarily of glycerolipids with embedded proteins which function as receptors, transporters, and enzymes to prevent free diffusion of hydrophilic molecules between the cellular organelles, and they control diffusion of substances in and out of cells (Buchanan et al., 2000; Dörmann, 1995; Spector and Yorek, 1985). Phospholipids are the most abundant membrane lipids composed of two hydrophobic fatty acid glycerol tails, a phosphate group, and a polar head group (Eyster, 2007). Two hydrophobic fatty

acid tails are oriented toward the inside of the membrane to avoid facing cellular water fluid and a polar head group is located outside of the membrane facing cellular fluid, thus forming the bilayer (Graham et al., 2003).

Plants are constantly exposed to both abiotic and biotic stresses and their lipids are associated with their responses to these stresses. For example, unsaturated fatty acid (linolenic acid) levels in chloroplast membranes affect membrane fluidity and the ability of a plant to tolerate abnormal temperatures (Iba, 2002). Linolenic acid is also involved in protein modifications in plants under heat stress (Yamauchi et al., 2008). Furthermore, jasmonic acid is one of the well-known, lipid-derived signal molecules involved in plant wound responses and plant disease (Shah, 2005). Enzymatic activity on plant lipids also helps them respond to stresses. For instance, phospholipase D (PLD) hydrolyzes phospholipids in membranes to generate phosphatidic acid (PA). The activity of PLD regulates a variety of diverse plant processes including freezing tolerance and programmed cell death.

Various stresses affect lipid composition in plant membranes. To adapt to cold stress, plants change the composition of membrane lipids to increase the amount of unsaturated phospholipids (Buchanan et al, 2000). Welti et al. (2002) showed that freezing and cold stresses induce a decline of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylglycerol (PG) but induce an increase in phosphatidic acid (PA) and lysophospholipids. Moellering et al. (2010) suggest that there is more lipid remodeling in the outer chloroplast membrane in freezing-tolerant plants compared to some freezing-sensitive plants. *SENSITIVE TO FREEZING 2* (*SFR 2*) is a gene that encodes a galactolipid remodeling enzyme of the outer chloroplast envelope,

and it transfers a galactosyl residue from monogalactolipid to different galactolipid accepters to form oligogalactolipids and diacylglycerol. The activity of *SFR 2* removes monogalactolipids from the envelope membrane and induces modification of the ratio of bilayer to non-bilayer membrane lipids to change organelle volume and stabilize membranes during freezing (Moellering et al., 2010). Zang et al. (2008) showed that thylakoid membrane lipid composition of drought resistant tomato has higher unsaturated phospholipid compared to wild type tomato. There were low 18:2 lipid amounts and high 18:3 lipid amounts in drought resistant tomato. The conclusion is that plants change the lipid composition in their membranes to acclimate to various stresses and there are different lipid compositions between wild-type plants and those with tolerance or resistance to the stress.

Because of its parasitic nature, *P. tritici-repentis* undoubtedly interacts with the lipids in wheat leaves. The activity of the tan spot fungus brings it into contact with lipids in the wheat plant when ascospores or conidia germinate by forming a germ tube under free moisture when they land on wheat leaves. The germ tube produces a penetration peg which facilitates penetration of the epidermal cell. Infection can be either direct or indirect, such as through stomata, and the penetration peg inside the plant forms a vesicle. Intracellular fungal hyphae grow and expand into other epidermal and mesophyll cells. Although *P. tritici-repentis* is intimately associated with lipids in wheat leaves, the interaction between the fungus and lipids has not been well characterized.

The toxins produced by *P. tritici-repentis* induce damage of cellular organelles and, therefore, may affect lipid profiles (Loughman and Deverall, 1986, Wegulo 2011). Manning and Ciuffetti (2005) suggest that Ptr ToxA is internalized in only sensitive

wheat cultivars and, once internalized, it localizes in the cytoplasm chloroplasts. The Ptr ToxA protein is able to cross the plant plasma membrane from the appoplastic space to the interior of the plant cell in the absence of the pathogen. The pathology of Ptr ToxA is plant cell death. Chlorosis of host leaves develops in response to Ptr ToxB due to inhibition of photosynthesis. The development of chlorosis leads to the photooxidation of the chlorophyll molecule as illuminated thylakoid membranes become unable to dissipate excess excitation energy (Stelkov et al., 1998). Kim et al. (2010) report that Ptr ToxB inhibits photosynthesis in toxin-sensitive wheat lines and suggest that it induces alterations of the proteome level in host metabolism. Ciuffetti el al. (2010) report that Ptr ToxA leads to a light-dependent accumulation of reactive oxygen species that correlate with the presence of necrosis and modify photosystem I and photosystem II in the absence of light.

Based upon the results summarized above, toxins produced by *P. tritici-repentis* are localized in chloroplasts and interact with host tissues. They result in inhibition of photosynthesis and induce chlorosis in wheat leaves. Because these toxins interact with the host in chloroplasts, and affect membranes in wheat leaves, a working hypothesis for this research was that there are likely different lipid compositions between diseased and healthy wheat cultivars. Therefore, one goal of the research was to determine if infection by *P. tritici-repentis* affects the lipid profiles in wheat leaves. A second goal was to determine if there was a difference in lipid profiles among cultivars susceptible or resistant to tan spot.

Materials and Methods

Influence of tan spot on lipid profiles.

Plant material for experiment #1. Wheat (*Triticum aestivum* L.) seeds were grown for one month in the greenhouse (20-28°C) in racks holding 2.5 × 13 cm plastic tubes (Stuewe and Sons, Tangent, OR) filled with a mixture of steam-sterilized soil and vermiculate (50:50). There were 48 treatments arranged in a randomized complete block design with five replications. An experimental unit was a single plant growing in a tube. Treatments included three resistant and three susceptible cultivars, with or without inoculation with *P. tritici-repentis*, and four harvest times after inoculation. Resistant cultivars were Betty, Jagger, and Karl 92 and susceptible cultivars were Larned, Newton, and TAM 105 (Table 1). Harvest times were 2, 4, 6, or 8 days after inoculation. The experiment was conducted twice.

Inoculum production and inoculation. Spores were produced by placing 0.5-cm^2 mycelial plugs of *P. tritici-repentis* from one-fourth-strength potato-dextrose agar (1/4 PDA) in the center of plates of V-8 agar (150 ml V-8 juice, 3 g $CaCO_3$, 15 g agar, 850 ml distilled water). Plates were incubated in the dark at 21-24°C for 5 days until the colony reached about 5 cm in diameter. Aerial mycelium was knocked down with a sterile, bent-glass rod and plates were incubated in the light (about 30 $\mu E\ s^{-1}\ m^{-2}$) at 21-24°C for 12 h to produce conidiophores and then in the dark at 16°C for 12 h to produce conidia. Thirty five milliliters of a spore suspension (about 10,000/ml) were applied per 30 × 60 cm rack holding two replications (96 tubes). Leaves were allowed to dry to stick spores to the leaves and the plants then placed into a mist chamber to maintain continual

leaf wetness for 48 h at 20-28°C. After the mist treatment, plants were returned to the greenhouse bench.

Disease rating, harvest, and processing. At each sample time, leaves were rated for percentage leaf area displaying chlorosis and/or necrosis (Raymond et al., 1985). After rating for leaf area affected by disease, lipid extraction was carried out according to the protocols published by the Kansas Lipidomics Research Center (http://www.k-state.edu/lipid/lipidomics/index.htm, Welti et al., 2002). The first and second leaves of each plant were removed, quickly cut with scissors into 1-cm pieces, and immersed in 6 ml preheated (75°C) isopropanol with 0.01% butylated hydroxytoluene (BHT). The extraction solvent was in a 50-ml glass tube with a lined screw cap. Leaf pieces were incubated in the 75°C isopropanol for at least 30 min. Three milliliters chloroform and 1 ml water were then added to each tube and the tube vortexed. Tubes were then agitated in a shaking incubator at room temperature for 1 h. The lipid extracts were transferred to another glass tube using a glass pipette. Four more extractions of lipid using 4 ml chloroform/methanol (2:1) with 0.01% BHT were carried out with shaking for 5 h or overnight until the leaves of the sample became white. Every sample had 5 extractions, including the one with the isopropanol. Samples were backwashed by adding 1 ml 1 M KCl to the combined extract, vortexing, centrifuging (10 min at 1000 rpm), and the upper phase removed. A second backwash involved adding 2 ml water and repeating the rinse cycle above. All tubes were then evaporated under nitrogen. After complete evaporation, the extract was dissolved in 1 ml chloroform. All extracts were stored at -75°C until analyzed for lipids. The remaining plant tissues were dried in an oven (105°C) overnight and then weighed (mg) to determine the dry extracted tissue weight.

Quantification of lipids using mass spectrometry. An aliquot from the dissolved extract in 1 ml chloroform was used for mass spectrometry lipid analysis. For analysis, 150-300 µl extract, dependent upon leaf dry weight, were combined with chloroform/methanol/300 mM ammonium acetate in water and internal standards. The ratio of solvent and internal standards was described previously (Devaiah et al., 2006; Welti, et al., 2002). The lipid extracts were analyzed by a triple quadrupole mass spectrometer MS/MS (API 4000, Applied Biosystem, Foster City, CA). Injections to the mass spectrometer were at the rate of 30 µl/min using an autosampler (LC Mini PAL, CTC Analytics AG, Zwingen, Switzerland) fitted with a needle.

Lipid profiles in resistant and susceptible cultivars (experiment #2).

Twenty winter wheat cultivars were selected based on Kansas State University extension ratings and ranged from resistant to susceptible to tan spot (Table 1). Seeds were grown in the greenhouse as described above for 1 month. A single seed was sown in each tube. The design was a randomized complete block with 20 treatments (cultivars) and 5 replications (3 plants per replication).

For lipid extraction, the first five extractions were the same as described above. After five chloroform/methanol based extractions, 4 ml hexane were added per tube and the tube incubated on a heating block at 60℃ for 15 min. The hexane solvent was then transferred to the chloroform/methanol extracts. The above procedure was repeated three more times. Evaporation, drying, and weighing of leaves followed as described above.

Data analysis. Data processing was carried out using custom script and Applied Biosystems Analyst software. The amounts of lipid species were calculated using the software program Excel and the LipidomeDB Data Calculation Environment (DCE)

(http://lipidome.bcf.ku.edu:9000/Lipidomics). Values were presented as nmol/mg dry tissue as derived as a percentage of the mass spectrometer signal in reference to known standards. Means were compared using analysis of variance (ANOVA) followed by Fisher's protected least significant difference (LSD) at *P*=0.05. To determine the effect of time, linear models were fit to data using SAS (SAS Institute, Cary, NC) and the resultant slopes were compared (*P*=0.05). When the slopes of two lines were not significantly different, the equal-slopes model was used to compare the estimates of the intercepts (*P*=0.05). For analysis of data from the experiment with 20 cultivars, linear regression was used to determine the relationship between the amount of lipid and cultivar rating to tan spot.

Results

Lipids detected in wheat leaves (experiment #1). Two galactolipids and nine phospholipids were identified by the mass spectrometer in extracts from wheat leaves (Fig. 1). The galactolipids were monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) and the phospholipids were phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidic acid (PA), lysophosphatidylcholine (LysoPC), lysophosphatidylethanolamine (LysoPE) and lysophosphatidylglycerol (LysoPG). The galatctolipids were the major lipid components in wheat leaves making up 89% of all lipid signals while the phospholipid classes were in relatively small amounts (Fig. 1). PC (7% of the signal) was the most abundant lipid within the phospholipid class. For both galactolipids, MGDG and DGDG, the major molecular species was 36:6 (di18:3) which

consists of two linolenic acid (18:3) moieties (Appendix 1). Similar results in lipid amounts were obtained in the repeat experiment.

Lipid profiles for healthy and diseased plants (experiment #1). When data for all cultivars were combined, there were significant differences between non-inoculated (healthy) and diseased plants (Table 2). In inoculated plants, the amounts of lipid for both glycolipids (DGDG and MGDG) were significantly (P<0.05) lower compared with those plants that were non-inoculated. Similarly, some identified phospholipids showed a statistical difference in amount between the healthy and diseased treatments (Table 2). The amounts of PG, PE, and PS in the healthy treatment were higher than those in the diseased treatment, but the amounts of LysoPC and PA in the healthy treatment were less than those in the diseased treatment. The rest of phospholipids (LysoPG, LysoPE, PC and PI) showed the same level between healthy and diseased treatments. Similar results were obtained in the repeat experiment except that there was no significant difference in the amounts of PE between healthy and diseased treatments, LysoPG was higher the healthy treatment, and PS was higher in the diseased treatment.

Lipid profiles for resistant and susceptible cultivars (experiment #1). In the analysis using ANOVA, only data from non-inoculated treatments were used to compare the resistant and susceptible treatments. Data from the three resistant cultivars were combined for analysis and compared with data from a combination of the three susceptible cultivars. Both of the glycolipids (DGDG and MGDG) had significantly (P<0.05) higher amounts in the resistant compared with the susceptible treatment (Table 3). Most phospholipids, except for PI, showed the same level in resistant and susceptible cultivars. PI was at higher amounts in the resistant cultivars compared with the

susceptible cultivars (Table 3). There were no lipids which were in higher amounts in susceptible cultivars. Similar results were obtained in the repeat experiment except LysoPC and PC were statistically higher in resistant cultivars and PI was at the same level in resistant and susceptible cultivars.

In another analysis, the effects of time and inoculation on resistant and susceptible cultivars were considered using linear regression. The slopes for inoculated plants of resistant cultivars had a significantly (*P*=0.0013) slower rate of decay for MGDG compared with susceptible cultivars (Fig. 2, Table 4, R+ vs. S+) with rates of -1.49 and -10.49 nmol/mg/day, respectively. A similar difference (*P*=0.0010) in rate of decay was observed for DGDG (Fig. 3, Table 4, R+ vs. S+) with slopes of -1.41 and -5.25 nmol/mg/day, respectively.

When comparing the lines for non-inoculated plants, none of the slopes for MGDG or DGDG were significantly different from zero indicating no significant change in lipid amount over time (Figs. 2 and 3). Similarly, the slopes for inoculated, resistant cultivars were not significantly different from those for non-inoculated, resistant or non-inoculated, susceptible cultivars (Table 4, R+ vs. R- and S-). However, as was observed with results using ANOVA (Table 3), the resistant cultivars consistently had higher amounts of these glycolipids compared with susceptible cultivars as indicated by the significantly higher estimates of the intercepts (Table 4, R- vs. S-). For MGDG, the comparison was 139.1 with 119.5 (*P*=0.123) and for DGDG, the comparison was 72.34 with 58.99 (*P*=0.0040).

Correlation of lipid amount with tan spot rating (experiment #2). Results from the ANOVA for the initial experiment (experiment #1) with six cultivars (Table 3)

showed that resistant cultivars contained different lipid profiles compared to the susceptible cultivars. Therefore, a second, expanded experiment involving 20 cultivars was conducted to corroborate that preliminary finding. The amounts of the major lipids were regressed against the disease phenotype rating for the cultivars. The cultivars had a range of reaction to tan spot from resistant to highly susceptible (Table 1). Figure 4 shows a significant (P<0.0001) negative correlation between the amount of MGDG and the extension rating. As the extension rating increased, the amount of MGDG decreased. There was a similar significant (P<0.0001) negative correlation between the amount of DGDG and tan spot extension rating; the higher the rating, the lower the amount of DGDG (Fig. 5).

Discussion

The results presented here give evidence that a biotic stress can profoundly affect lipid profiles in plants. They are the first to document the influence of the wheat leaf spot disease tan spot on lipids in wheat leaves. When comparing lipid profiles in inoculated vs. healthy plants, tan spot resulted in significant changes in lipid profiles in most of the detected lipids (Table 2). Healthy wheat leaves had more of the lipids MGDG, DGDG, PG, PE, and PS. For MGDG and DGDG, comprising 89% of the lipids in wheat leaves, differences of 24.7 and 19.7% were measured, respectively (Table 2). Furthermore, reductions of over 50% were observed for both lipids in inoculated, susceptible cultivars 8 days after inoculation (Figs. 2 and 3, S+). It is unknown whether the fungus itself degraded the lipids or whether it induced plant enzymes to degrade the lipids. Further research is needed to elucidate the answer to that question.

Plants interact with the biotic and abiotic environments and have systems to protect themselves against stresses. When exposed to stresses, their survival often depends on how fast they recognize and response to these stresses (Maffei et al., 2007). The plasma membrane of a plant cell is often the first component where plants interact with environmental stresses. Early events in the interaction between plants and environmental stresses can involve activities such as a kinase signal transduction pathway, phytohormones, and the production of reactive oxygen species in the plasma membrane (Maffei et al., 2007). During these interactions, the composition of lipids in the cell membrane is changed.

There have been many studies involving changes in lipid profiles in plant cell membranes. However, most previous studies have focused on changes in plant lipid composition due to abiotic stresses. Important findings in membrane biology concern the relationship between lipid composition and how plants adjust to temperature stress (Wolter et al., 1992). In this regard, unsaturated fatty acids are linked to biochemical and physiological changes in plants exposed to chilling injury. Murata et al. (1992) proposed a hypothesis that the level of unsaturated phosphatidylglycerol (PG) in chloroplast membranes determines the chilling sensitivity of plant species.

In this research, some lipids were observed to increase in amount in diseased compared with healthy leaves. One notable result was the increase of the level of PA observed in diseased tissue (Table.2, PA). This probably occurred because PA is generated by the activity of phospholipase D which hydrolyses other lipid molecules such as PC, PE, and PG. PA has many functions such as a signaling molecule in plant defense pathways under abiotic and biotic stresses. Previous studies have reported the increase of

PA under various stresses; drought, salinity, wounding, cold, and pathogen infection. For example, the level of PA increased four times within 5 minutes in wounded tomato seedlings (Lee et al., 1997). Welti et al. (2002) reported that freezing induced an increase in PA and a decrease in PC. In this study, the level of PE and PG decreased in diseased leaves; however, a significant reduction in PE was not detected in the repeat experiment. Therefore, further investigation is needed to confirm this finding.

Results shown here are the first to correlate the amount of lipid moieties in wheat leaves with resistance level to tan spot. Wheat cultivars resistant or susceptible to tan spot showed different lipid profiles. Resistant cultivars had more MGDG, DGDG and PI (Table 3). The other lipids showed the same amount of lipid in resistant and susceptible cultivars. For the major lipid species MGDG and DGDG, resistant cultivars had 15.0 and 14.3% more, respectively, than susceptible cultivars (Table 3). Similar results were observed when looking at the data over time. For non-inoculated treatments, the slopes of the lines for the galactolipids MGDG and DGDG were the same for resistant and susceptible cultivars and not significantly different from zero (Figs. 2 and 3, Table 4, R- vs. S-). This indicates that the levels did not change over time in healthy leaves. However, the estimates of the intercepts showed that there were higher ($P<0.05$) levels of those lipids in the resistant cultivars (Table 4, 139.1 vs. 119.5 for MGDG and 72.34 vs. 58.99 for DGDG). Similarly, the rates of reduction due to tan spot for the galactolipids were significantly different between resistant and susceptible cultivars. Resistant cultivars had a significantly slower loss (larger slope) of MGDG and DGDG compared with susceptible cultivars (Figs. 2 and 3, Table 4, R+ vs. S+). For MGDG, the slope of susceptible cultivars was -10.49, significantly ($P=0.0013$) lower than the slope of

resistant cultivars (-1.49) (Fig. 2, Table 4). Similarly, for DGDG, the slope for susceptible cultivars (-5.25) was significantly (P=0.0010) lower than that for resistance cultivars (-1.41) (Fig. 3, Table 4). Therefore, these results suggest that lipids in susceptible wheat cultivars are influenced by tan spot more than those in resistant cultivars and the disease results in faster degradation of galactolipids in susceptible cultivars.

The experiment using 20 cultivars corroborated the above finding of higher levels of the major lipids in resistant cultivars. There was a significant (P<0.0001) negative linear relationship between the amounts of MGDG and DGDG in non-inoculated wheat leaves and the level of resistance to tan spot (Figs. 4 and 5). As the level of resistance increased (lower rating number), the level of MGDG and DGDG also increased. Using calculations from the linear equations, cultivars with a rating of 1 would have 66.1% more MGDG and 52.7% more DGDG than cultivars with a rating of 9 (Figs. 4 and 5). MGDG and DGDG are major membrane constituents of chloroplasts and most abundant in plant leaves. They are indispensible for efficiency of photosynthetic light reactions. For example, Jarvis et al. (2000) reported that MGDG synthase activity in the Arabidopsis mutant *mgd1* was reduced by 50% relative to that of the wild-type, thus reducing the amount of MGDG and chlorophyll. Similarly, mutant plants with *dgd 1* contained 10% of the wild-type amount of DGDG and the mutant plants showed a strong reduction of photosynthetic capacity (Dörmann et al., 1995; Härtel et al., 1997; and Reinfarth et al., 1997).

Three kinds of host specific toxins are involved in the pathogenesis of tan spot diseases. These toxins move into chloroplasts and interact with thylakoid membranes so

that inhibition of photosynthesis in wheat leaves occurs (Stelkov et al., 1998; Manning and Ciuffetti, 2005; Kim et al., 2010). Therefore, it may be that the toxins are responsible for the change in lipid profiles documented in this research. However, further research is needed to document this possibility.

In conclusion, data presented here are the first to quantify the effects of tan spot on lipid profiles in wheat leaves. Data from time-course experiments indicate that tan spot significantly reduced the amount of many lipids including the major lipids MGDG and DGDG in leaves. This reduction was at a much higher rate for susceptible cultivars compared with resistant ones. Furthermore, data showed that cultivars resistant to tan spot have different lipid profiles when compared with susceptible cultivars. Resistant cultivars had more MGDG and DGDG than susceptible ones, even in non-inoculated leaves. These findings are indirect evidence that the amounts of some lipids in wheat leaves are a determinant in the resistance response of cultivars to tan spot. However, further research is needed to corroborate this conclusion.

Acknowledgements

This work was supported by the Kansas Wheat Commission and the Kansas Agricultural Experiment Station. Contribution number KAES #XX-XXX-J from the Kansas Agricultural Experiment Station.

Literature Cited

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. 2002. Membrane transport of small molecules and the electrical properties of membranes. Page 651 -694 in: Molecular biology of the cell. Garland Science. New York.

Ballance, G.M., Lamari, L., and Bernier, C.C. 1989. Purification and characterization of a host-selective necrosis toxin from *Pyrenophora tritici-repentis*. Physiological and Molecular Plant Pathology 35:203–213.

Ballance, G.M., Lamari, L., Kowatsch, R., and Bernier, C.C. 1996. Cloning, expression and occurrence of the gene encoding the Ptr necrosis toxin from *Pyrenophora tritici-repentis*. Molecular Plant Pathology. http://www.bspp.org.uk/mppol/1996/1209ballance/.

Buchanan B.B.,Gruissem,W., and Jones, R.L.2000. Lipids. Page 456-527: in Biochemistry & molecular biology of plants. American society of Plant Physiologists. Rockville.

Ciuffetti, L.M., Tuori, R.P., and Gaventa, J.M. 1997. A single gene encodes a selective toxin causal to the development of tan spot of wheat. Plant Cell 9:135–144.

Ciuffetti, L.M., and Tuori, R.P. 1999. Advances in the Characterization of *the Pyrenophora tritici-repentis*-Wheat Interaction. Phytopathology 89:444-449

Ciuffetti, L.M., Manning, V.A., Pandelova, I., Betts, M.F., and Martinez, J.P. 2010. Host-selective toxins, Ptr ToxA and Ptr ToxB, as necrotrophic effectors in the *Pyrenophora tritici-repentis*–wheat interaction. New Phytologist 187:911-919.

Christie, W.W. "What is a lipid?". Lipidlibrary.aocs.org. Updated Nov. 8th 2011. http://lipidlibrary.aocs.org/Lipids/whatlip/index.htm#def

Devaiah S.P., Roth M.R., Baughman E., Li M., Tamura P., Jeannotte R., Welti R., and Wang, X. 2006. Quantitative profiling of polar glycerolipid species fromorgans of wild-type Arabidopsis and a phospholipase Dalpha1 knockout mutant. Phytochemistry 67: 1907-1924

De Wolf, E. D., Bockus, W. W., and Whitworth, J. R. 2011. Wheat Variety Disease and Insect Ratings 2011. Kansas Cooperative Extension Service publication MF-991. 4 pp.

De Wolf E.D., Effertz R.J., Al S., Francl L.J. 1998. Vistas of tan spot research. Canadian Journal of Plant Pathology 20: 349–370.

Dörmann, P., Hoffmann-Benning, S., Balbo, I., and Benning, C. 1995. Isolation and characterization of an Arabidopsis mutant deficient in the thylakoid lipid digalactosyl diacylglycerol. Plant Cell 7:1801–1810.

Effertz, R.J., Meinhardt, S.W., Anderson, J.A., Jordahl, J.G., and Francl, L.J. 2002. Identification of a chlorosis-inducing toxin from *Pyrenophora tritici-repentis* and the chromosomal location of an insensitivity locus in wheat. Phytopathology 92:527–533.

Eyster, K.M. 2007. The membrane and lipids as integral participants in signal transduction: lipid signal transduction for the non-lipid biochemist. Advances in Physiology Education 31:5-16.

Gamba, F.M., Lamari, L., and Brule-Babel, A. L. 1998. Inheritance of race-specific necrotic and chlorotic reactions induced by *Pyrenophora tritici-repentis* in hexaploid wheats. Canadian Journal of Plant Pathology 20:401–407.

Graham, L.E., Graham, J.M., and Wilcox, L.W. 2003.Four types of primary compounds are the molecules of life. Pages 55-59 in: Plant biology. Pearson Education. Upper Saddle River.

Härtel, H., Lokstein, H., Dörmann, P., Grimm, B., and Benning, C. 1997. Changes in the composition of the photosynthetic apparatus in the galactolipid-deficient *dgd1* mutant of *Arabidopsis thaliana*. Plant Physiology 115:1175-1184.

Iba, K. 2002. Acclimative response to temperature stress in higher plants: approaches of gene engineering for temperature tolerance. Annual Review Plant Biology 53:225-45.

Jarvis, P., Dörmann, P., Peto, C.A., Lutes, J., Benning, C., and Chory, J. 2000. Galactolipid deficiency and abnormal chloroplast development in the *Arabidopsis MGD synthase 1* mutant. Proc. Natl. Acad. Sci. 97:8175–8179.

Kim, Y. M., Bouras, N., Kav, N. N., and Strelkov, S. E. 2010. Inhibition of photosynthesis and modification of the wheat leaf proteome by Ptr ToxB: a host-specific toxin from the fungal pathogen *Pyrenophora tritici-repentis*. Proteomics 10:2911-2926.

Lamari, L., and Bernier, C. C. 1989. Evaluation of wheat lines and cultivars to tan spot (*Pyrenophora tritici-repentis*) based on lesion type. Canadian Journal of Plant Pathology 11:49-56.

Lamari, L., and Strelkov, S. E. 2010. The wheat/*Pyrenophora tritici-repentis* interaction: progress towards an understanding of tan spot disease. Canadian Journal of Plant Pathology 32:4-10.

Lee, S., Suh, S., Kim, S., Crain, R. C., Kwak, J. M., Nam, H.-G., and Lee, Y. 1997. Systemic elevation of phosphatidic acid and lysophospholipid levels in wounded plants. The Plant Journal 12:547–556.

Loughman, R., and Deverall, B. J. 1986. Experiments with excised lesions from wheat leaves infected by *Pyrenophora tritici-repentis*. Australasian Plant Pathology 18:90-93.

Maffei, M. E., Mithofer, A., and Boland, W. 2007. Before gene expression: early events in plant–insect interaction. Trends in Plant Science 12:310-316.

Manning, V. A., and Ciuffetti, L. M. 2005. Localization of Ptr ToxA produced by *Pyrenophora tritici-repentis* reveals protein import into wheat mesophyll cells. Plant Cell 17:3203–3212.

McMullen, M., and T. Adhikari. 2009. Fungal leaf spot diseases of wheat: tan spot, stagonospora nodorum blotch and septoria tritici blotch. PP-1249. North Dakota State University Extension Service.

McMullen, M.P. 2010. Tan spot (yellow leaf spot). Pages 82-84 in: Compendium of Wheat Diseases and Pests. W.W. Bockus, R.L. Bowden, R.M. Hunger, W.L. Morrill, T.D. Murray, and R.W. Smiley, eds. American Phytopathological Society, St. Paul, MN

Moellering, E. R., Muthan, B., and Benning, C. 2010. Freezing tolerance in plants requires lipid remodeling at the outer chloroplast membrane. Science 330:226-228.

Murata N., Ishizaki-Nishizawa, O., Higashi, S., Hayashi, H., Tasaka, Y., and Nishida, I. 1992. Genetically engineered alteration in the chilling sensitivity of plants. Nature 356:313-326.

Raymond, P. J., Bockus, W. W., and Norman, B. L. 1985. Tan spot of winter wheat: Procedures to determine host response. Phytopathology 75:686-690.

Reifarth, F., Christen, G., Seeliger, A.G., Dörmann, P., Benning, C., and Renger, G. 1997. Modification of the water oxidizing complex in leaves of the *dgd1* mutant of *Arabidopsis thaliana* deficient in the galactolipid digalactosyldiacylglycerol. Biochemistry 36:11769-11776.

Shabeer, A., and Bockus, W.W. 1988. Tan spot effects on yield and yield components relative to growth stage in winter wheat. Plant Disease 72:599-602.

Shah, J. 2005. Lipids, lipases, and lipid-modifying enzymes in plant disease resistance. Annual Review Phytopathology 43:229-226.

Singh, P. K., and Hughes, G. R. 2005. Genetic control of resistance to tan necrosis induced by *Pyrenophora tritici-repentis*, races 1 and 2, in spring and winter wheat genotypes. Phytopathology 95:172-177.

Spector A.A., Yorek M.A. 1985. Membrane lipid composition and cellular function. Journal of Lipid Research 26: 1015–1035.

Strelkov, S. E., Lamari, L., and Balance, G. M. 1998. Induced chlorophyll degradation by a chlorosis toxin from *Pyrenophora tritici-repentis*. Canadian Journal of Plant Pathology 20:428–435.

Strelkov, S. E., and Lamari, L. 2003. Host-parasite interaction in tan spot [*Pyrenophora tritici-repentis*] of wheat. Canadian Journal of Plant Pathology 25:339–349.

Tomas, A., Feng, G. H., Reeck, G. R., Bockus, W. W., and Leach, J. E. 1990. Purification of a cultivar-specific toxin from *Pyrenophora tritici-repentis*, causal agent of tan spot of wheat. Mol. Plant Microbe Interact. 3:221–224.

Tuori, R. P., Wolpert, T. J., and Ciuffetti, L. M. 1995. Purification and immunological characterization of toxic components from cultures of *Pyrenophora tritici-repentis*. Mol. Plant Microbe Interact. 8:41–48.

USDA-NASS. 2010. Wheat varieties. Online. USDA-NASS, Kansas Field Office, Dept. of Agric., Topeka, KS.

Voet, D., Voet, J. G., and Pratt, C. W. 2006. Biological membranes and lipids. Page 234-281 in: Fundamentals of Biochemistry. Wiley. Hoboken.

Welti, R., Li, W., 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 D alpha in freezing-induced lipid changes in Arabidopsis. Journal of Biochemistry 277:31994-32002.

Wegulo, S.N. 2011. Tan spot of cereals. The Plant Health Instructor. http://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Pages/TanSpot.aspx

Weselake, R.J. 2005. Storage lipids. Page 162-221 in: Plant lipids; biology, utilization and manipulation. Murphy, D.J. ed. Blackwell. Oxford.

Wolter, F. P., Schmidt, R., and Heinz, E. 1992. Chilling sensitivity of Arabidopsis thaliana with genetically engineered membrane lipids. The European Molecular Biology Organization Journal 11:4685-4692.

Yamauchi, Y., Furutera, A., Seki, K., Toyoda, Y., Tanaka, K., and Sugimoto, Y. 2008. Malondialdehyde generated from peroxidized linolenic acid causes protein modification in heat-stressed plants. Plant Physiology Biochemistry 46:786-793.

Zhang, C., Liu, Y., Sun, X., Qian, W., Zhang, D., and Qiu, B. 2008. Characterization of a specific interaction between IP-L, a tobacco protein localized in the thylakoid membranes, and Tomato mosaic virus coat protein. Biochemical and Biophysical Research Communications 374:253-257.

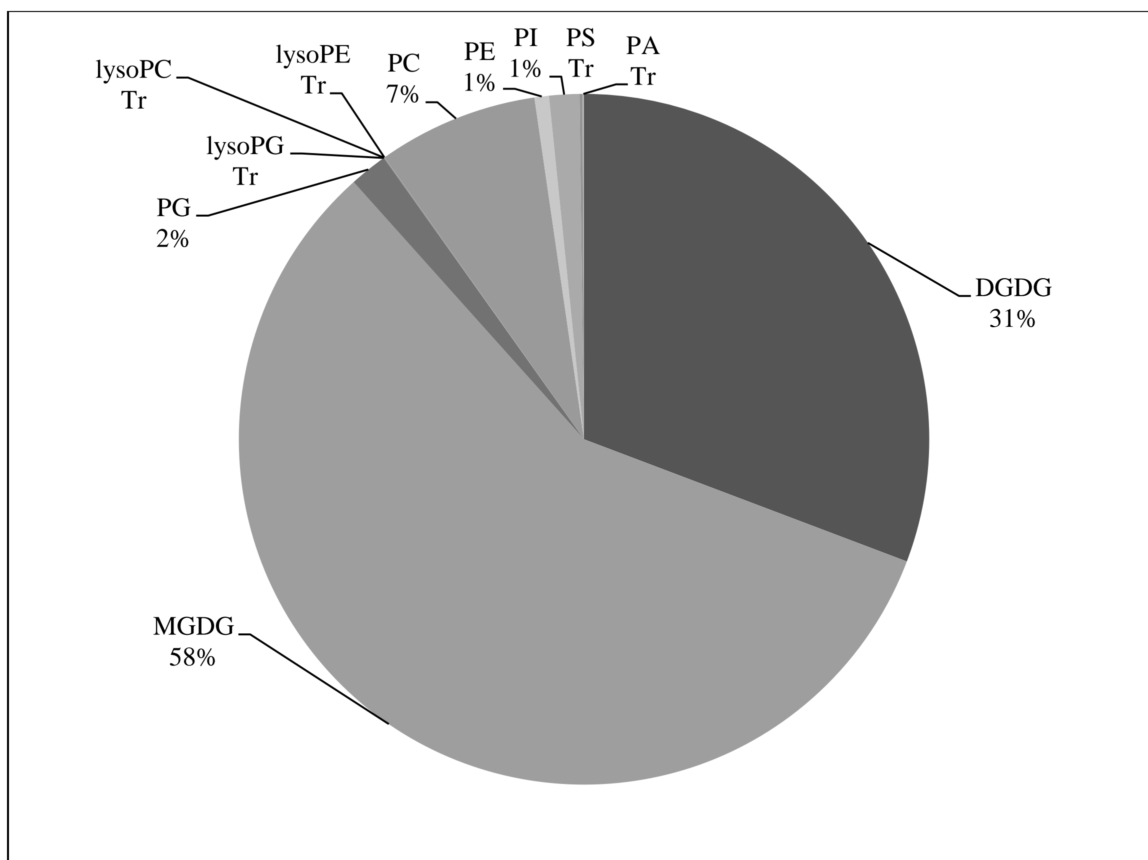


Fig. 1. Total percentage of each lipid detected in non-inoculated wheat leaves; average of plants that were 30, 32. 34, and 36 days old. Llipids with "Tr" indicate detection at very low levels ("Trace"). Abbreviations: MGDG = monogalactosyldiacylglycerol; DGDG = digalactosyldiacylglycerol; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PG = phosphatidylglycerol; PI = phosphatidylinositol; PS = phosphatidylserine; PA = phosphatidic acid; lysoPC = lysophosphatidylcholine; lysoPE = lysophosphatidylethanolamine; and lysoPG = lysophosphatidylglycerol.

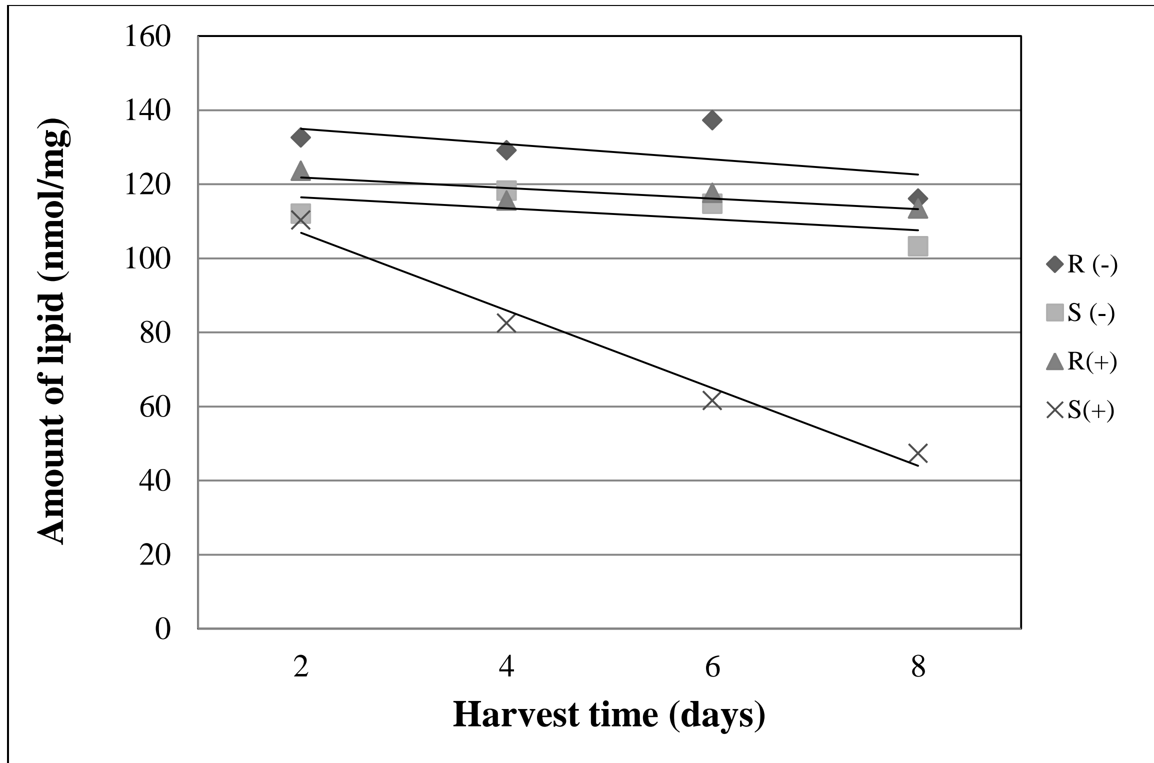


Fig. 2. Amount of monogalactosyldiacylglycerol (MGDG) in wheat leaves over time. One-month-old plants were inoculated or left non-inoculated at day 0. Abbreviations are: R- = average of three resistant wheat cultivars without inoculation; S- = average of three susceptible cultivars without inoculation; R+ = resistant cultivars with inoculation; and S+ = susceptible cultivars with inoculation. Each data point is the mean of three cultivars each with five replications. Equations for the trend lines and *P* values for significance of the slopes different from zero are as follows: R- , Y = -2.06 X + 139.1 (*P* = 0.2826); S-, Y = -1.49 X + 119.5 (*P* = 0.4370); R+, Y = -1.49 X + 125.1 (*P* = 0.4360); and S+, Y = -10.49 X + 127.9 (*P* = <0.0001).

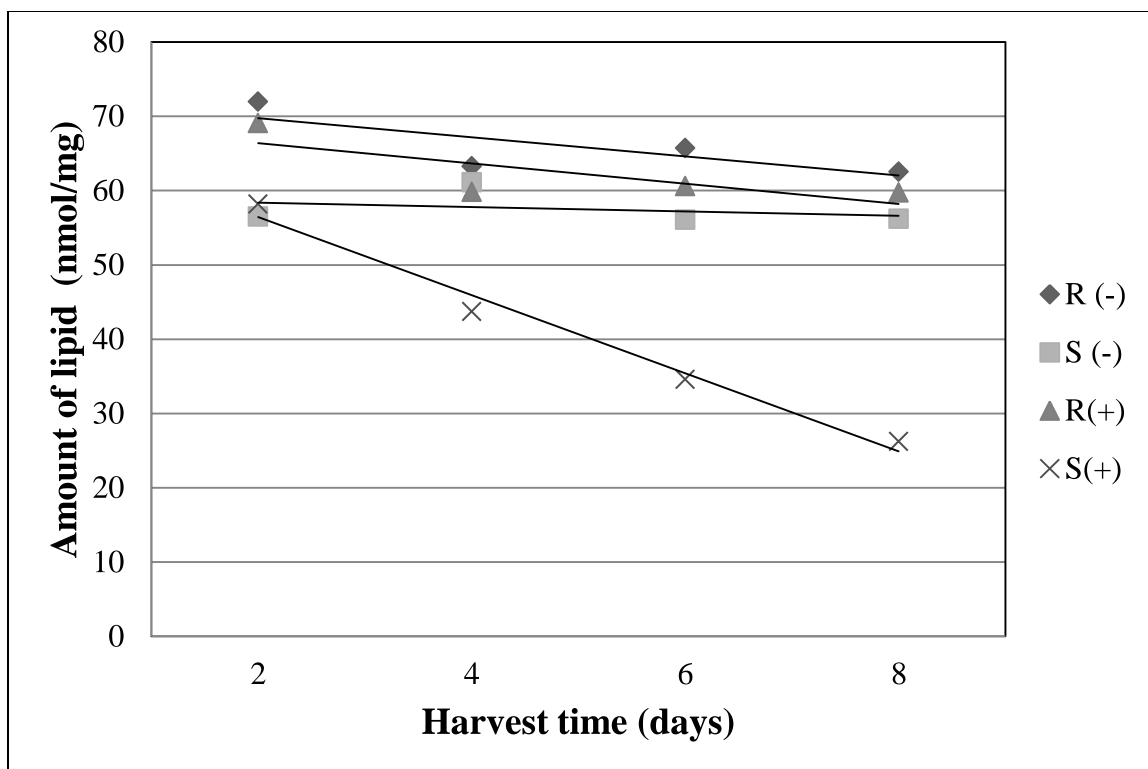


Fig. 3. Amount of digalactosyldiacylglycerol (DGDG) in wheat leaves over time. One-month-old plants were inoculated or left non-inoculated at day 0. Abbreviations are: R- = average of three resistant wheat cultivars without inoculation; S- = average of three susceptible cultivars without inoculation; R+ = resistant cultivars with inoculation; and S+ = susceptible cultivars with inoculation. Each data point is the mean of five replications. Equations for the trend lines and *P* values for significance of the slopes different from zero are as follows: R- , Y = -1.29 X + 72.34 (*P* = 0.1088); S-, Y = -0.300 X + 58.99 (*P* = 0.7071); R+, Y = -1.41 X + 69.42 (*P* = 0.0808); and S+, Y = -5.25 X + 66.93 (*P* = <0.0001).

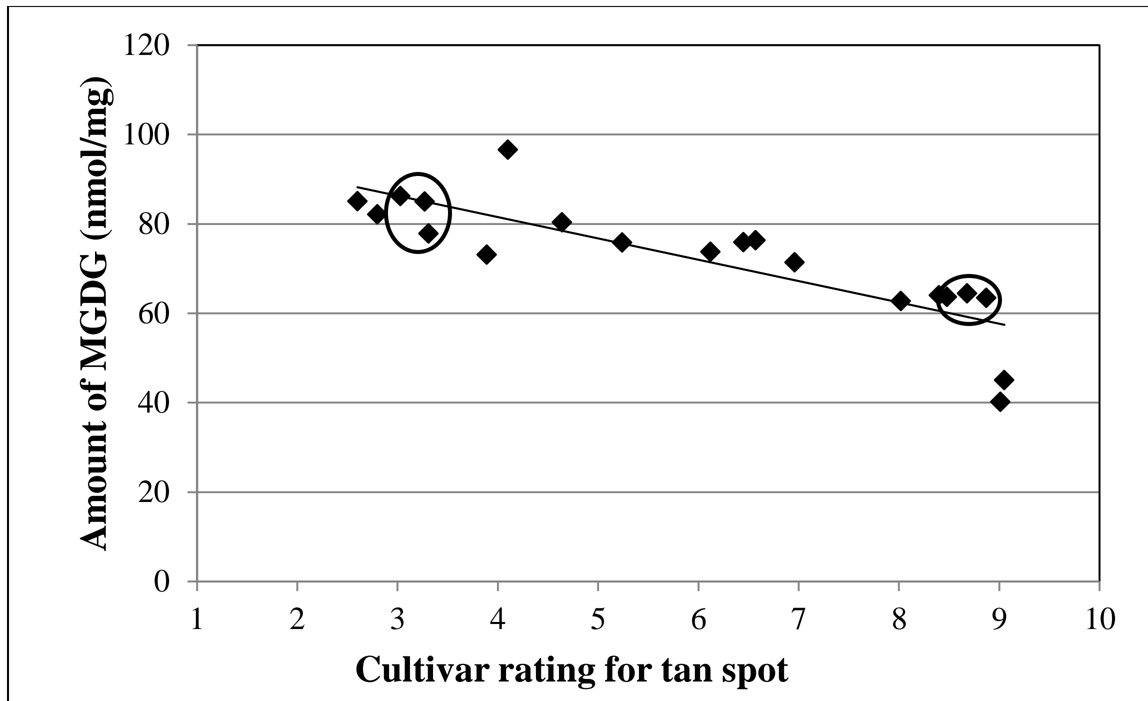


Fig.4. Amount of monogalactosyldiacylglycerol (MGDG) versus the level of resistance to tan spot for non-inoculated, 28-day-old seedling leaves of 20 winter wheat cultivars. Rating values are on a 1-9 scale where 1 = highly resistant to tan spot and 9 = highly susceptible. Each data point is the mean of five replications for a single cultivar. Linear equation is: Y = -4.77 X + 100.63 (Adjusted $R^2 = 0.6815$, $N = 20$, and $P < 0.0001$). The three resistant and three susceptible cultivars that were used in experiment #1 are circled.

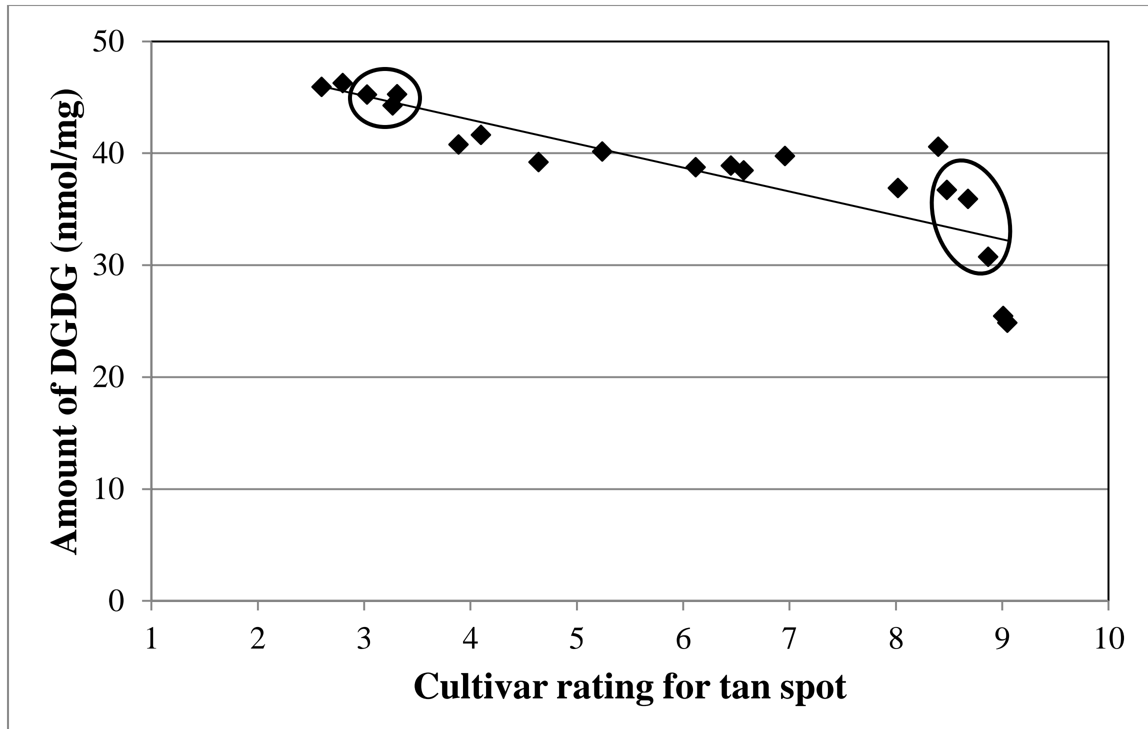


Fig.5. Amount of digalactosyldiacylglycerol (DGDG) versus the level of resistance to tan spot for non-inoculated, 28-day-old seedling leaves of 20 winter wheat cultivars. Rating values are on a 1-9 scale where 1 = highly resistant to tan spot and 9 = highly susceptible. Each data point is the mean of five replications for a single cultivar. Linear equation is: Y = -2.13 X + 51.52 (Adjusted $R^2 = 0.7041$, $N = 20$, and $P < 0.0001$). The three resistant and three susceptible cultivars that were used in experiment #1 are circled.

Table1. Reaction of 20 selected winter wheat cultivars to tan spot

Entry No.	Cultivar	Tan spot rating[a]
1	Red Chief	2.60
2	Heyne	2.80
3	Betty	3.03
4	Karl 92	3.27
5	Jagger	3.31
6	2137	3.89
7	Victory	4.10
8	Overley	4.64
9	Wesley	5.24
10	Protection CL	6.12
11	Onaga	6.45
12	Jagalene	6.57
13	Abilene	6.96
14	Ike	8.02
15	2180	8.40
16	Newton	8.48
17	Larned	8.68
18	TAM 105	8.87
19	Arkan	9.01
20	Stanton	9.05

[a]1-to-9 scale where 1 = highly resistant and 9 = highly susceptible. Values are the means of at least five replicated phenotyping experiments (see De Wolf et al., 2011 and Bockus, *unpublished*).

Table 2. Comparison of amounts (nmol/mg dry tissue) of lipids in healthy (non-inoculated) and tan spot-infected wheat leaves

Lipid species[a]	Healthy	Diseased
MGDG	120.4[bc] a	96.53 b
DGDG	61.69 a	51.53 b
PG	3.54 a	2.98 b
LysoPG	0.003 a	0.005 a
LysoPC	0.03 b	0.05 a
LysoPE	0.01 a	0.01 a
PC	11.46a	12.59 a
PE	1.27 a	1.13 b
PI	2.47 a	2.37 a
PS	0.27 a	0.24 b
PA	0.06 b	0.11 a

[a]Abbreviations: MGDG = monogalactosyldiacylglycerol; DGDG = digalactosyldiacylglycerol; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PG = phosphatidylglycerol; PI = phosphatidylinositol; PS = phosphatidylserine; PA = phosphatidic acid; lysoPC = lysophosphatidylcholine; lysoPE = lysophosphatidylethanolamine; and lysoPG = phosphatidylglycerol.

[b] Values within a row, when followed by the same letter, are not significantly different according to ANOVA followed by Fisher's protected least significant difference (P<0.05).

[c] Values are the mean of six cultivars averaged across four harvest dates with five replications per cultivar per harvest date.

Table 3. Comparison of lipid amounts (nmol/mg dry tissue) between non-inoculated winter wheat cultivars resistant and susceptible to tan spot

Lipid species[a]	Resistant	Susceptible
MGDG	128.8[bc] a	112.0 b
DGDG	65.9 a	57.5 b
PG	3.55 a	3.54 a
LysoPG	0.004 a	0.002 a
LysoPC	0.033 a	0.031 a
LysoPE	0.015 a	0.014 a
PC	12.7 a	10.2 a
PE	1.32 a	1.21 a
PI	2.64 a	2.30 b
PS	0.28 a	0.26 a
PA	0.04 a	0.07 a

[a]Abbreviations: MGDG = monogalactosyldiacylglycerol; DGDG = digalactosyldiacylglycerol; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PG = phosphatidylglycerol; PI = phosphatidylinositol; PS = phosphatidylserine; PA = phosphatidic acid; lysoPC = lysophosphatidylcholine; lysoPE = lysophosphatidylethanolamine; and lysoPG = phosphatidylglycerol.
[b] Values within a row, when followed by the same letter, are not significantly different according to ANOVA followed by Fisher's protected least significant difference (*P*<0.05).

[c] Values are the mean of three cultivars in each category (Resistant or Susceptible) averaged across four harvest dates with five replications per cultivar per harvest date.

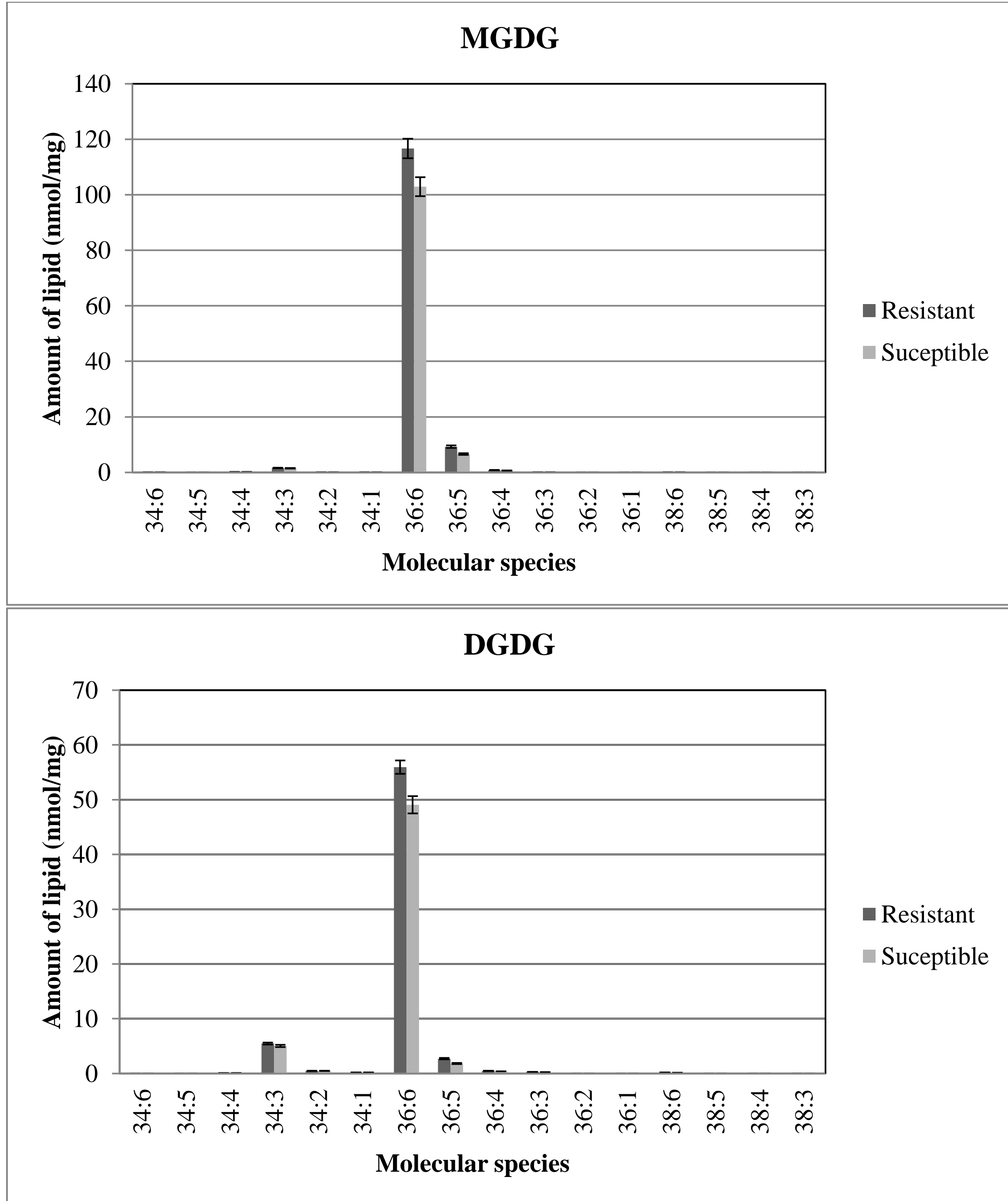
Table 4. Statistical *P* values for the comparison of slopes (in parentheses) and estimates of the intercepts (in parentheses) for the amount of MGDG[a] or DGDG regressed against harvest time for wheat cultivars resistant and susceptible to tan spot (lines shown in Figs. 2 and 3).

MGDG			
Comparison of slopes	S-[b] (-1.49)	R+ (-1.49)	S+ (-10.49)
R- (-2.06)	0.8321	0.8331	0.0025
S- (-1.49)	-	0.9990	0.0013
R+ (-1.49)	-	-	0.0013
Comparison of intercepts[c]			
R- vs. S- (139.1 vs. 119.5)	0.0123		
R+ vs. R- (125.1 vs. 139.1)	0.0919		
R+ vs. S- (125.1 vs. 119.5)	0.3933		
DGDG			
Comparison of slopes	S- (-0.300)	R+ (-1.41)	S+ (-5.25)
R- (-1.29)	0.3811	0.9174	0.0007
S- (-0.300)	-	0.3277	<0.0001
R+ (-1.41)	-	-	0.0010
Comparison of intercepts[c]			
R- vs. S- (72.34 vs. 58.99)	0.0040		
R+ vs. R- (69.42 vs. 72.34)	0.2184		
R+ vs. S- (69.42 vs. 58.99)	0.0875		

[a] Abbreviations: MGDG = monogalactosyldiacylglycerol; and DGDG = digalactosyldiacylglycerol.
[b] R- = wheat cultivars resistant to tan spot without inoculation; S- = susceptible cultivars without inoculation; R+ = resistant cultivars with inoculation; and S+ = susceptible cultivars with inoculation. Slopes and estimates of the intercepts are shown in parentheses.
[c] The estimates of the intercepts were only compared for those pairings where the slopes of the lines were not significantly different.

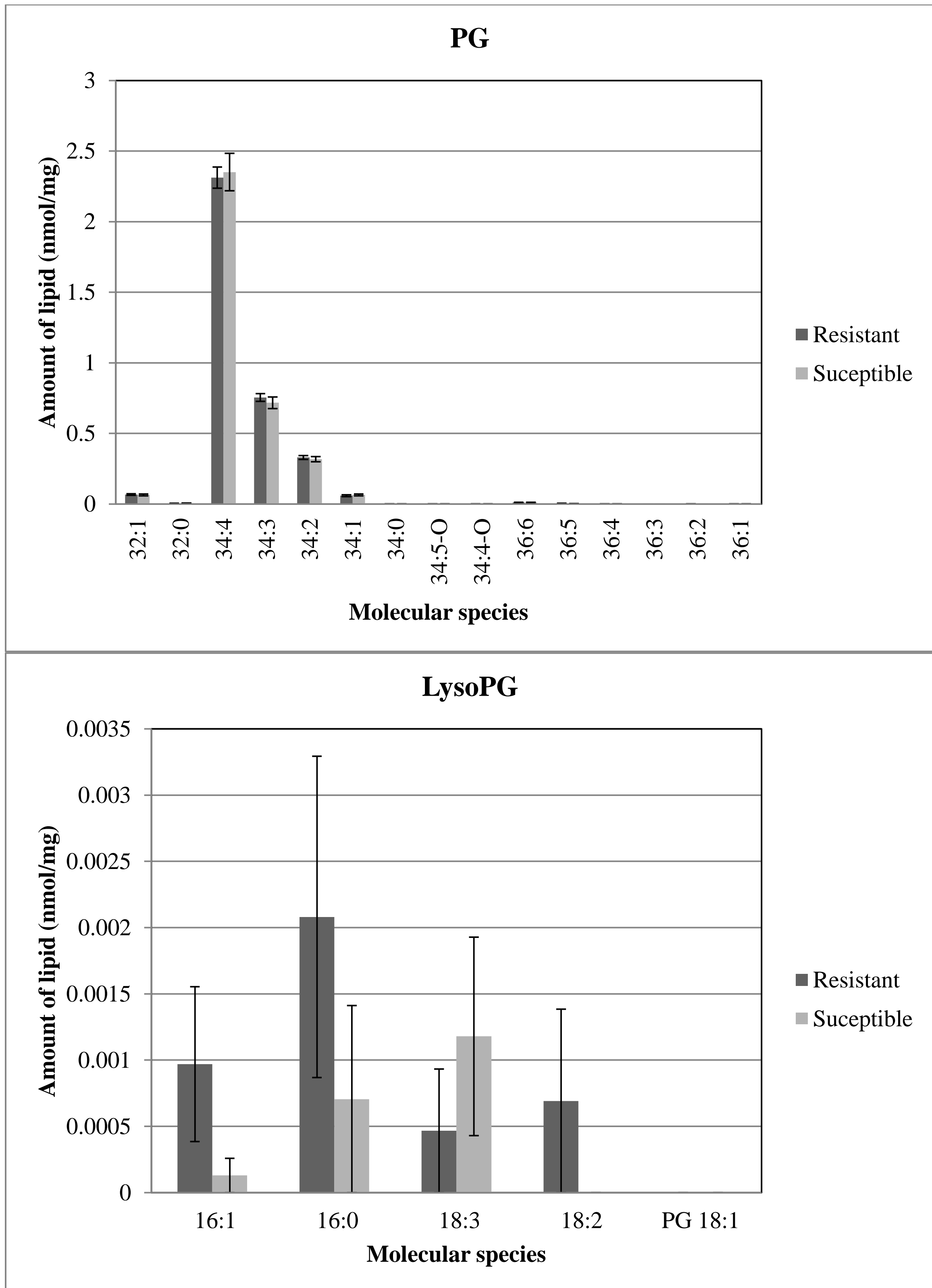
Appendices

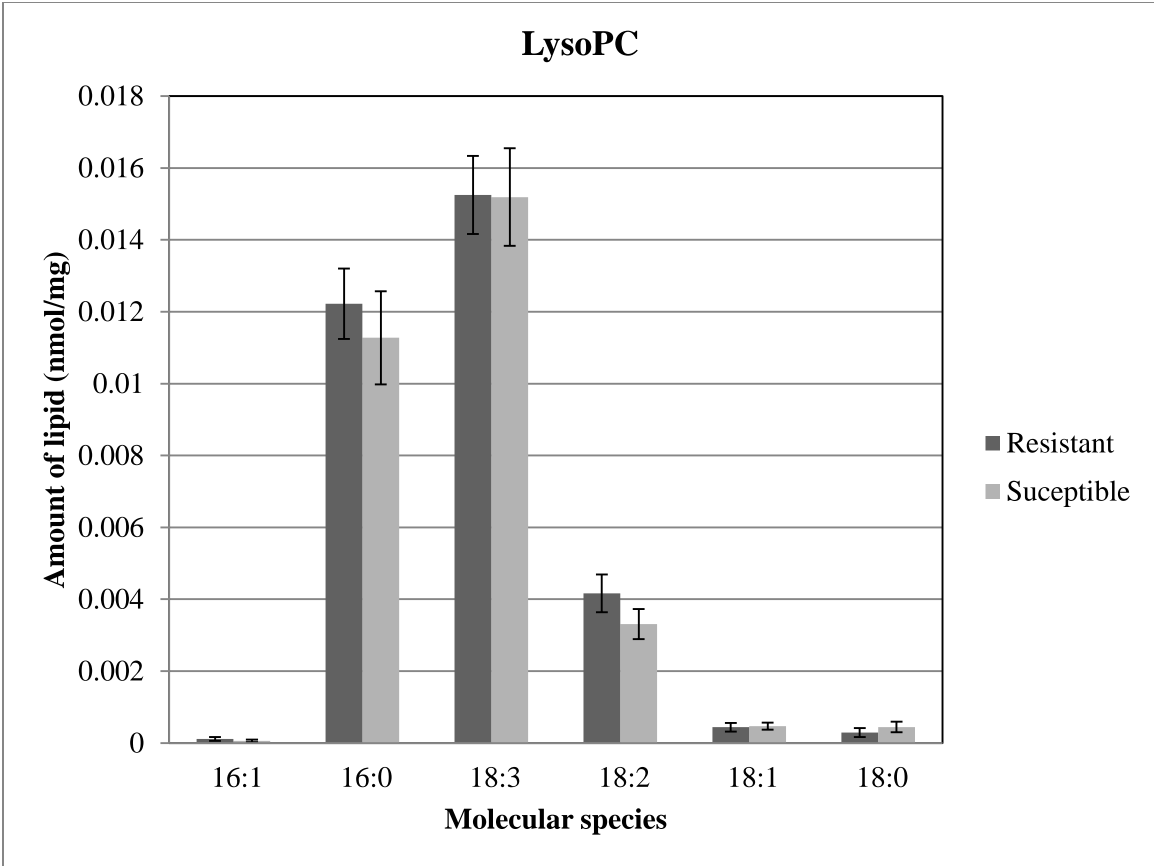
Appendix 1. Profiles of major lipid molecular species in noninoculated resistant and susceptible wheat leaves averaged across all harvest dates.

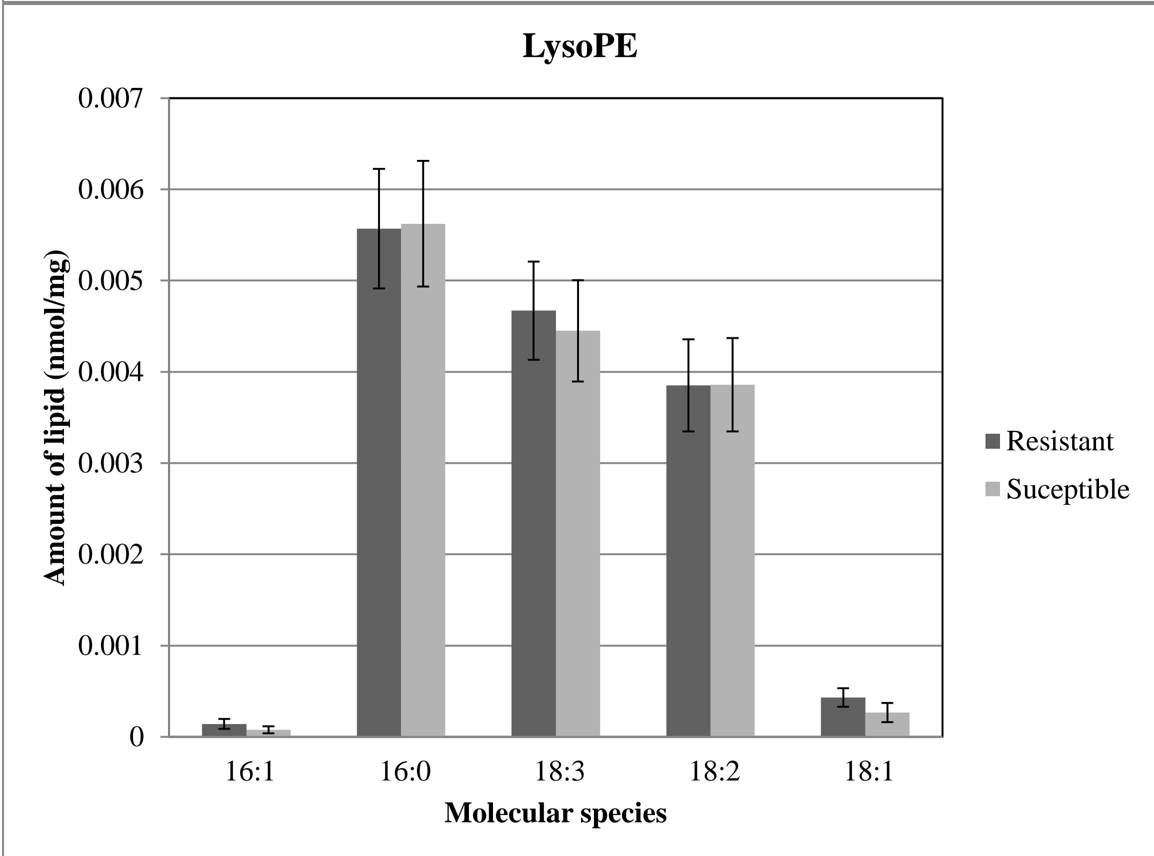


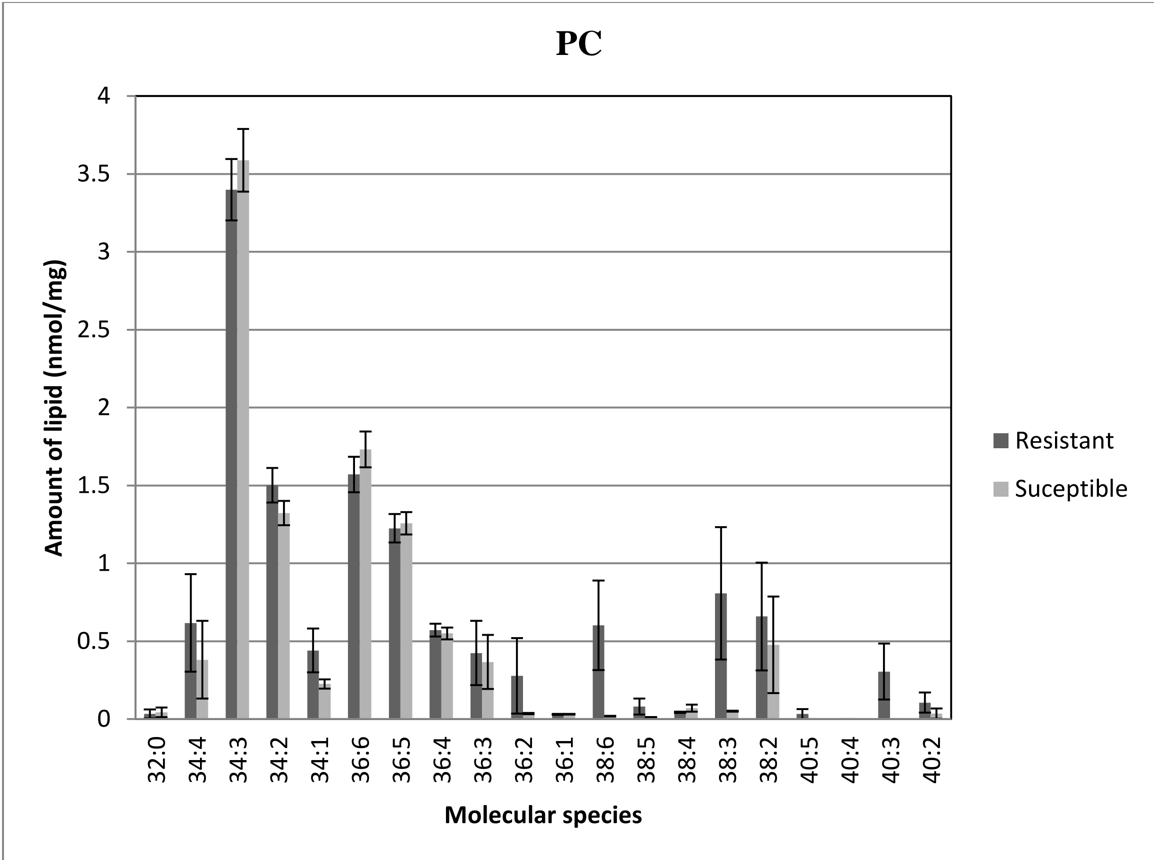
Abbreviations: MGDG = monogalactosyldiacylglycerol; and DGDG = digalactosyldiacylglycerol.

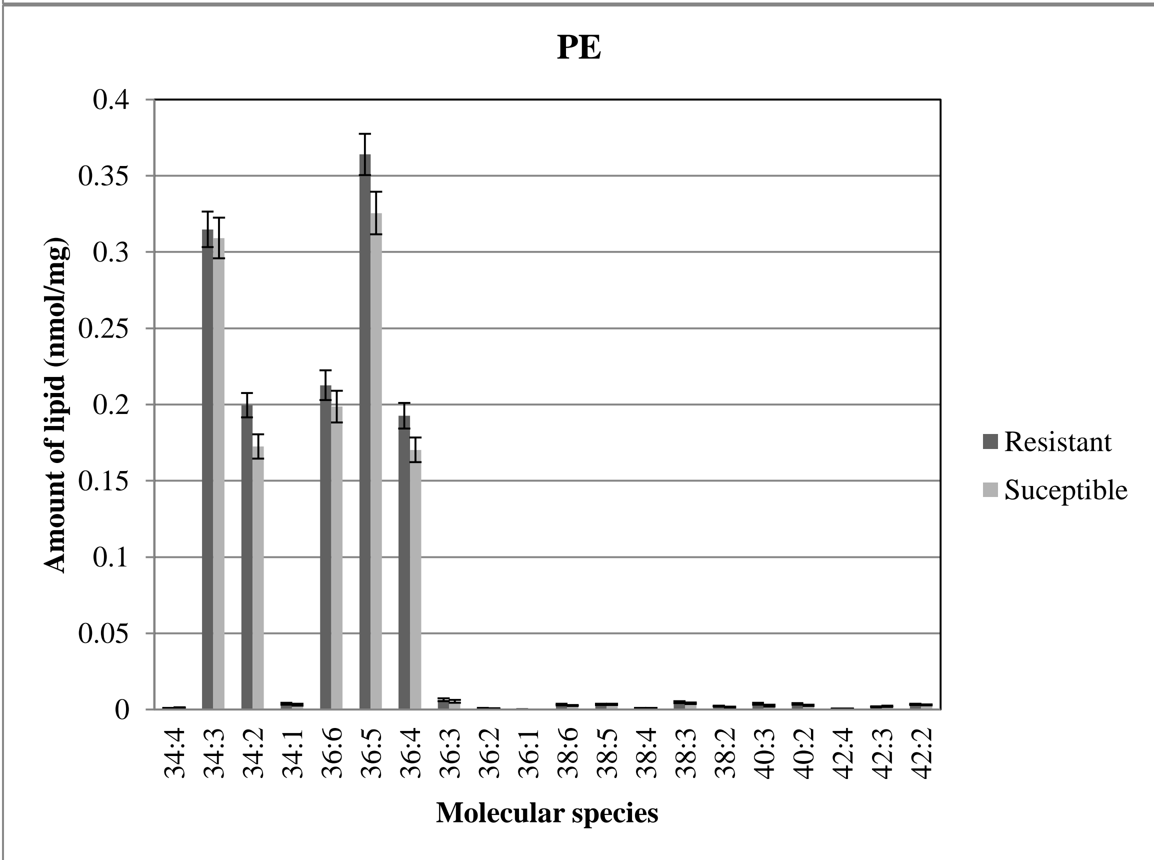
Appendix 2. Profiles of minor lipid molecular species in non-inoculated wheat leaves averaged across all harvest dates.

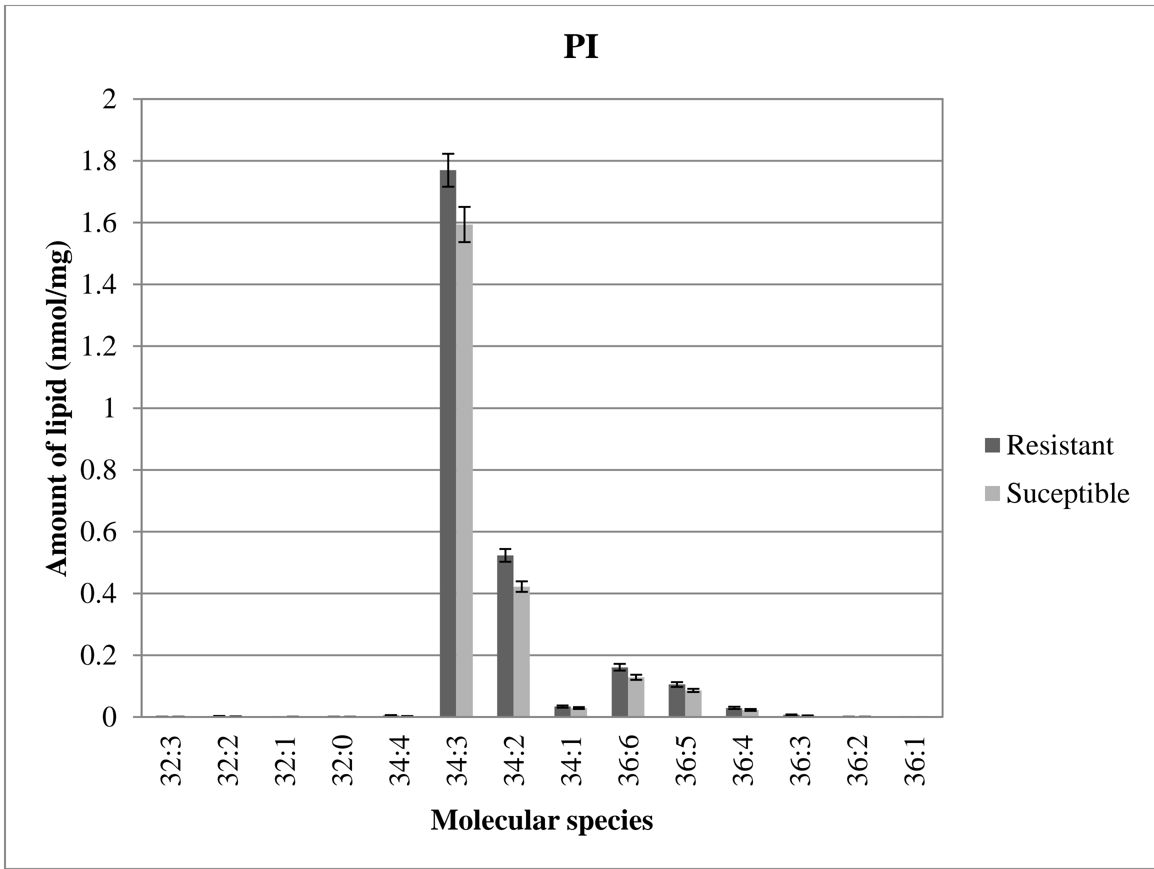


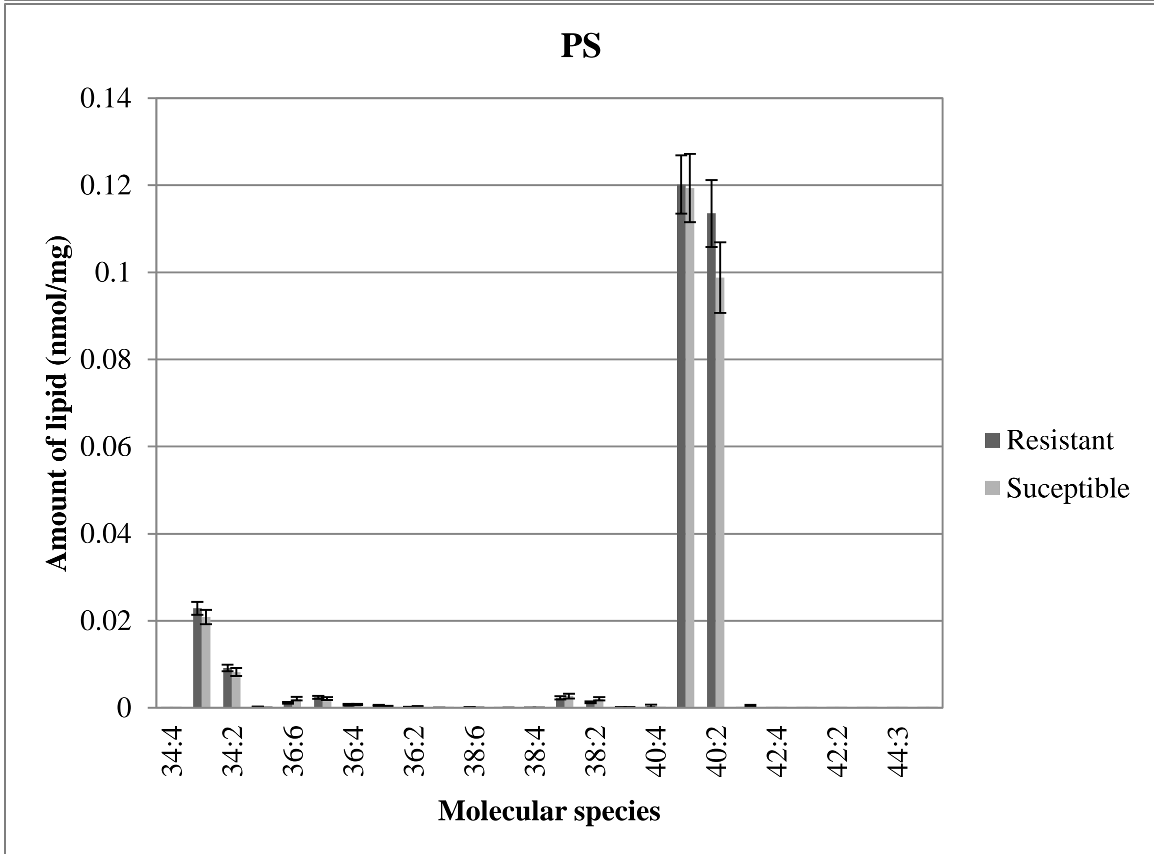

LysoPC
0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
Amount of lipid (nmol/mg)
16:1
16:0
18:3
18:2
18:1
18:0
Molecular species
Resistant
Suceptible

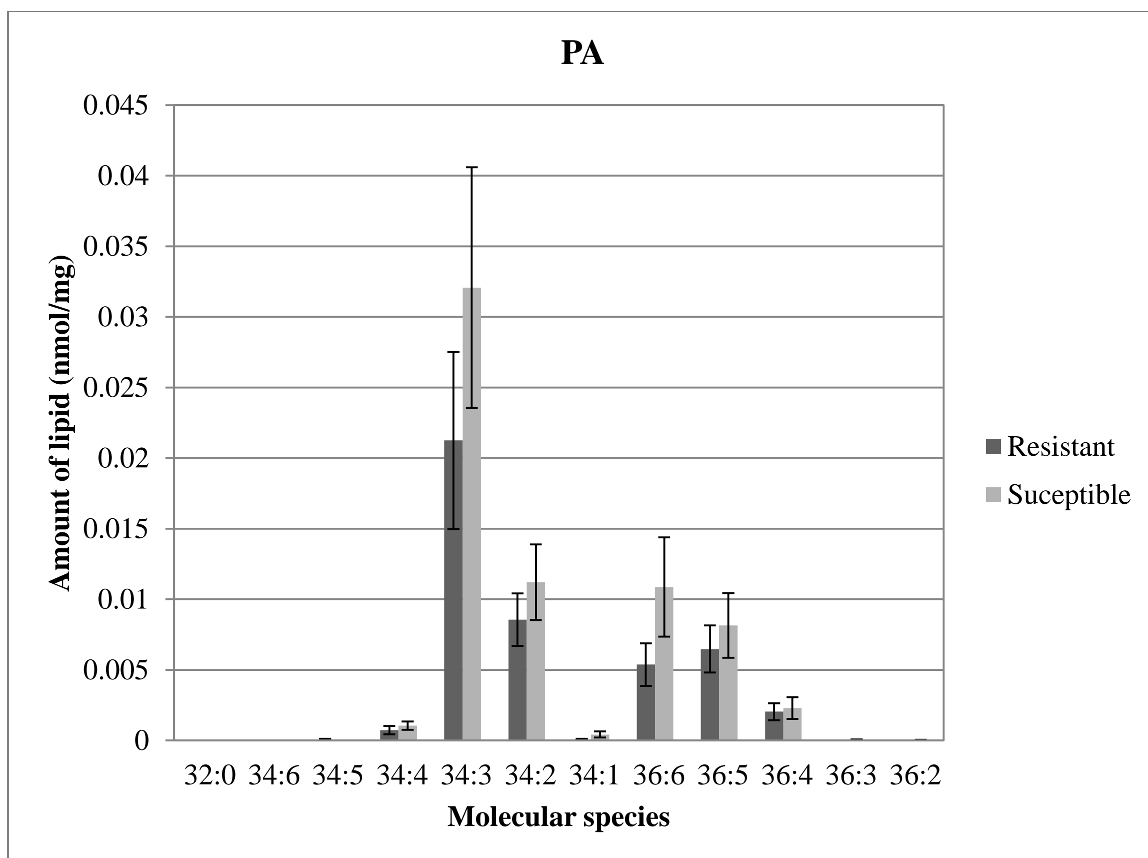

LysoPE
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
Amount of lipid (nmol/mg)
16:1
16:0
18:3
18:2
18:1
Molecular species
Resistant
Suceptible


PC
Amount of lipid (nmol/mg)
4
3.5
3
2.5
2
1.5
1
0.5
0
32:0
34:4
34:3
34:2
34:1
36:6
36:5
36:4
36:3
36:2
36:1
38:6
38:5
38:4
38:3
38:2
40:5
40:4
40:3
40:2
Molecular species
Resistant
Suceptible


PE
Amount of lipid (nmol/mg)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
34:4
34:3
34:2
34:1
36:6
36:5
36:4
36:3
36:2
36:1
38:6
38:5
38:4
38:3
38:2
40:3
40:2
42:4
42:3
42:2
Molecular species
Resistant
Suceptible


PI
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Amount of lipid (nmol/mg)
32:3
32:2
32:1
32:0
34:4
34:3
34:2
34:1
36:6
36:5
36:4
36:3
36:2
36:1
Molecular species
Resistant
Suceptible


PS
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Amount of lipid (nmol/mg)
34:4
34:2
36:6
36:4
36:2
38:6
38:4
38:2
40:4
40:2
42:4
42:2
44:3
Molecular species
Resistant
Suceptible



Each bar represents the mean of 120 samples; error bars are also shown. Abbreviations: PC = phosphatidylcholine; PE = phosphatidylethanolamine; PG = phosphatidylglycerol; PI = phosphatidylinositol; PS = phosphatidylserine; PA = phosphatidic acid; lysoPC = lysophosphatidylcholine; lysoPE = lysophosphatidylethanolamine; and lysoPG = lysophosphatidylglycerol.