Genes and genetic mechanisms contributing to fall armyworm resistance in maize

Marilyn L. Warburton[1], Sandra Woolfolk[1], Jessie Smith[1], Leigh Hawkins[1], Lina Castano-Duque[1], Matthew Lebar[1], and William Williams[1]

[1]USDA ARS

January 18, 2023

Abstract

Maize (Zea mays L.) is a crop of major economic and food security importance globally. The fall armyworm (FAW), Spodoptera frugiperda, can devastate entire maize crops, especially in countries or markets that do not allow the use of transgenic crops. Host-plant insect resistance is an economical and environmentally benign way to control FAW, and this study sought to identify maize lines, genes, and pathways that contribute to resistance to FAW. Of 289 maize lines phenotyped for FAW damage in artificially infested, replicated field trials over three years, 31 were identified with good levels of resistance that could donate FAW resistance into elite but susceptible hybrid parents. The 289 lines were genotyped by sequencing to provide SNP markers for a genome-wide association study (GWAS), followed by a metabolic pathway analysis using the Pathway Association Study Tool (PAST). GWAS identified 15 SNPs linked to 7 genes, and PAST identified multiple pathways, associated with FAW damage. Top pathways, and thus useful resistance mechanisms for further study, include hormone signaling pathways and the biosynthesis of carotenoids (particularly zeaxanthin), chlorophyll compounds, cuticular wax, known antibiosis agents, and 1,4-dihydroxy-2-naphthoate. Targeted metabolite analysis confirmed that maize genotypes with lower levels of FAW damage tend to have higher levels of chlorophyll a than genotypes with high FAW damage, which also tend to have lower levels of pheophytin, lutein, chlorophyll b and β-carotene. The list of resistant genotypes, and the results from the genetic, pathway, and metabolic study, can all contribute to efficient creation of FAW resistant cultivars.

To the Editors of the Plant Genome:

Our collaborative research project on Genome-wide Association Study of Fall Armyworm resistance in maize, followed by metabolic pathway analysis, has resulted in the manuscript presented here. This is the first work of its kind on this trait in maize, and the second on insects, and we believe it will be useful to plant breeders working on insect resistance, as well as geneticists and physiologists working to decipher complex traits in plant populations. We hope the reviewers and editors of The Plant Genome agree, and we look forward to hearing your suggestions on further improvement of our paper.

Sincerely yours, Marilyn Warburton and co-authors.

Core ideas:

- GWAS and metabolic pathway analysis identified genes and pathways associated with Fall Armyworm resistance.
- 31 maize lines with good resistance to Fall Armyworm were identified that can be useful donor lines.
- Pathways identified may provide evidence of new resistance mechanisms for direct selection or new research avenues.
- Measurement of targeted metabolites confirm pathway analysis results and provide further avenues of new research.

Genes and genetic mechanisms contributing to fall armyworm resistance in maize

Marilyn L. Warburton[1], Sandra W. Woolfolk[2], J. Spencer Smith[2], Leigh K. Hawkins[2], Lina Castano-Duque[3], Matthew D. Lebar[3], and W. Paul Williams[2]

[1]USDA ARS Plant Germplasm Introduction and Testing Research Unit, Pullman, WA

[2]USDA ARS Corn Host Plant Resistance Research Unit, Mississippi State, MS

[3]USDA ARS Food and Feed Safety Research Unit, New Orleans, LA

Abbreviations: BLUE (Best Linear Unbiased Estimator); CIMMYT (International Maize and Wheat Improvement Center, in Spanish); CHPRRU (Corn Host Plant Resistance Research Unit); FAW (fall armyworm); GBS (genotyping by sequencing); GLM (general linear model); GWAS (genome-wide association study); MAF (minor allele frequency); MLM (mixed linear model); PAST (pathway analysis study tool); QTL (quantitative trait loci).

Abstract

Maize (*Zea mays* L.) is a crop of major economic and food security importance globally. The fall armyworm (FAW), *Spodoptera frugiperda,* can devastate entire maize crops, especially in countries or markets that do not allow the use of transgenic crops. Host-plant insect resistance is an economical and environmentally benign way to control FAW, and this study sought to identify maize lines, genes, and pathways that contribute to resistance to FAW. Of 289 maize lines phenotyped for FAW damage in artificially infested, replicated field trials over three years, 31 were identified with good levels of resistance that could donate FAW resistance into elite but susceptible hybrid parents. The 289 lines were genotyped by sequencing to provide SNP markers for a genome-wide association study (GWAS), followed by a metabolic pathway analysis using the Pathway Association Study Tool (PAST). GWAS identified 15 SNPs linked to 7 genes, and PAST identified multiple pathways, associated with FAW damage. Top pathways, and thus useful resistance mechanisms for further study, include hormone signaling pathways and the biosynthesis of carotenoids (particularly zeaxanthin), chlorophyll compounds, cuticular wax, known antibiosis agents, and 1,4-dihydroxy-2-naphthoate. Targeted metabolite analysis confirmed that maize genotypes with lower levels of FAW damage tend to have higher levels of chlorophyll a than genotypes with high FAW damage, which also tend to have lower levels of pheophytin, lutein, chlorophyll b and β-carotene. The list of resistant genotypes, and the results from the genetic, pathway, and metabolic study, can all contribute to efficient creation of FAW resistant cultivars.

Introduction

Maize (*Zea mays* L.) provides food, feed, and industrial ingredients to the economy of the United States, worth many billions of dollars annually. It is a major staple food crop globally as well, and total grain production was 1,266 million tons in 2019 (World Data Atlas, Knoema.com). The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), originally evolved with maize in the tropical and subtropical regions of the Americas, and although it feeds on a broad range of host plants, it prefers gramineous plants including maize, and can devastate a maize crop in a short time (Overton et al., 2021). It has now spread to almost every country of the Americas, Africa, and Asia as an invasive and destructive species (Goergen et al., 2016; FAO 2021). This is of particular concern in Africa, where many countries do not allow the use of transgenic crops, removing some insect control options from farmers, but new genetic editing techniques may be more acceptable to the public (Turnbull et al., 2021).

Native resistance, or host-plant insect resistance conferred by naturally occurring maize genes, is an economical and environmentally benign way to control FAW. Maize lines containing native resistance genes were created in the 1970s through 1990s by the International Maize and Wheat Improvement Center (CIMMYT) in Mexico (Mihm et al., 1988). The USDA-ARS Corn Host Plant Resistance Research Unit developed and released several resistant temperate maize inbred lines; these include Mp496, Mp701-708, Mp713, Mp714, and Mp716 (Scott and Davis, 1981; Scott et al., 1982; Williams and Davis, 1980, 1982, 1984, 2000, 2002; Williams et al., 1990). Although high levels of FAW infestation are better controlled by transgenic hybrids

containing the Bt gene than conventional hybrids with native resistance, both transgenic and conventionally bred resistant maize hybrids are significantly less damaged by FAW feeding than non-resistant commercial hybrids (Williams et al., 1997).

Native resistance to insects in maize is generally quantitative in nature, governed by many genes, rather than a single gene conferring most or all of the resistance (McMullen et al., 2009). Quantitative trait loci (QTL) for FAW resistance in maize have been identified including one with a very large phenotypic effect in bin 9.03 (Brooks et al., 2007; Womack et al., 2018; 2020). Genetic mechanisms causing FAW resistance in maize can be due to morphological features such as structural barriers (waxes or tougher cell walls); antibiosis, or the creation of metabolites toxic or off-putting to the insects; or hormones that call insect predators (McMullen et al., 2009). A previous study by Davis et al. (1998) found that resistance in young plants was linked to thickness of the cuticle and the epidermal cell wall complex, which was nearly twice as thick in resistant than susceptible plants and contained more hemicellulose. In addition, the inner whorl tissue from the resistant inbreds were tougher than in the susceptible, and resistant hybrids completed the transition from juvenile to adult tissues (which are less palatable to insects) sooner than susceptible hybrids. FAW larvae feeding on maize plants in the whorl stage of growth feed primarily on the yellow-green portion of whorl leaves. Davis et al. (1999) compared the sizes of larvae fed the yellow-green leaves of resistant or susceptible hybrids, compared to the outer (green) or innermost (yellow-white) portions of the leaves. Larvae feeding on the susceptible hybrid were larger than those feeding on the resistant, and larvae feeding on the yellow-green leaves in resistant (and to a lesser extent, susceptible) plants were smaller compared to those feeding on other portions within the whorl. Thus, it is hypothesized that there are antibiosis compounds localized there, especially in resistant plants, that protect the growing point of the seedling plants (Williams et al., 1998).

Both QTL mapping and association mapping may identify genes and genomic regions associated with a trait of interest. A QTL mapping experiment will likely identify very long genomic regions associated with the trait and will only find the alleles that happen to be segregating in the two parents of the mapping population. Conversely, a genome-wide association study (GWAS) will identify far more regions associated with a trait, often precisely enough to narrow down to one or a few genes influencing the trait, but rarely identifies large effect genes for highly quantitative traits, and many genes may be missed (Flint-Garcia et al., 2005). A metabolic pathway analysis of the output of a GWAS study may remedy this, and may identify more useful genes than the GWAS, and these genes will be grouped into pathways of known function (Tang et al., 2017; Thrash et al., 2022; 2021). The spread of FAW outside its native range has increased the importance of identifying genes and regions of the maize genome contributing resistance to FAW both to facilitate breeding of non-GMO lines with FAW resistance, and to identify genes as candidates for gene editing. Thus, this study was undertaken to dissect the genetic basis and physiological mechanisms of resistance to FAW in maize. The objectives of this study were to identify resistant maize lines and SNPs associated with FAW resistance via GWAS; to use this information to identify metabolic pathways associated with FAW resistance via PAST; validate the presence of one or more of these metabolites in resistant vs. susceptible plants; to integrate these pathways into specific mechanisms used by maize plants as protection from FAW; and to compare these results to previous genomic and metabolomic studies of insect resistance in maize.

Materials and Methods

Germplasm and data generation

A panel of 289 diverse maize inbred lines was used for this study (Supplemental Table 1). The panel included both FAW resistant and susceptible lines from various breeding programs, some of which formed association panels published previously (Warburton et al., 2013; Flint-Garcia et al., 2005). These lines were genotyped and phenotyped as inbreds. They were grown at the R.R. Foil Plant Science Research Center, Mississippi State, Mississippi (MS) in 2019, 2020, and 2021. Seeds were planted in 4 m single-row plots with 0.97 m spacing between rows and thinned to 20 plants plot^{-1}. The experiment was planted in a randomized complete block design with two replications in 2019 and 2020 and three replications in 2021. Standard production practices were followed, and supplemental irrigation was applied as needed.

The fall armyworm neonate larvae used in this study were obtained from the laboratory colony at the insect rearing facility of the Corn Host Plant Resistance Research Unit (CHPRRU), USDA-ARS, Mississippi State. The larvae were reared using artificial diet following the procedure described by Davis (1989). Within 24 hours following the egg hatch, first instar larvae (neonates) were mixed with corn cob grits. The mixture was dispensed into the maize whorl utilizing a mechanical applicator (Wiseman et al., 1980). Each individual plant was infested with approximately 40 FAW neonates at the mid-whorl stage (V-7 of maize growth stage). All plants within experimental plots were rated individually to determine the leaf feeding damage caused by FAW. The damage was visually rated at 7 days and 14 days after infestation. The 7-day and 14-day leaf feeding ratings were conducted on a 0 (no visible leaf feeding damage) to 9 scale according to Davis et al. (1992; Supplemental Table 2). The 7-day ratings were conducted by one person and the 14-day ratings were conducted independently by two people.

The mean rating for each entry was calculated as an adjusted mean (Best Linear Unbiased Estimator; BLUE) using mixed model methodology in the PROC GLIMMIX procedure of SAS 9.4 (SAS Institute, Cary, NC) and can be found in Supplemental Table 1. For the 7-day ratings, the individual plants within a plot were treated as subsamples. Plot means were calculated within years treating genotype as a fixed effect and replication and genotype by replication as random effects. To combine the 7-day ratings over years, genotype was treated as a fixed effect and year, replication nested within year, year by genotype interaction, and genotype by replication nested in year were all treated as random effects. For the 14-day ratings two sets of individual plant ratings, one set per rater, were used as subsamples for each plot. Plot means were calculated similarly to the 7-day ratings, but additional model terms were added to account for the variance due to different raters. Within years, genotype was treated as a fixed effect and replication, rater, rater by replication, genotype by replication, and genotype by rater by replication were all treated as random effects. When the data was combined across three years, genotype was treated as a fixed effect and year, replication nested in year, rater, rater by replication nested in year, year by genotype interaction, year by rater interaction, genotype by replication nested in year, and rater by genotype by replication nested in year were all treated as random effects.

GWAS and pathway analyses

Genotyping of the inbred lines in the panel was done via Genotype by Sequencing (GBS) according to Elshire et al. (2011). Briefly, SNPs were extracted from raw GBS data using the Java pipeline for GBS Bioinformatics (Glaubitz et al. 2014). SNPs were aligned against the B73 reference genome, version 4. The GBS marker dataset was imputed and filtered by removing SNPs with a minor allele frequency (MAF) $<$ 6% and any SNP with $>$ 2 alleles, resulting in a total of 1,105,817 SNPs. Useful GBS data was successfully obtained from 281 of the inbred lines, which were used in the GWAS. A subset consisting of 8,000 SNPs with a low missing data rate ($<$ 7.5%) and a balanced allele frequency (MAF $>$ 40%) was extracted for calculation of population sub-structure (Q matrix) and linkage disequilibrium, and 148,000 unimputed SNPs were used to calculate the K matrix. K, Q, and LD were calculated in the same manner reported in Warburton et al., (2015). The software package TASSEL 3.0 (Bradbury et al., 2007) was used to perform the GWAS using the BLUE values of the 7-day and 14-day FAW ratings within and across years. The General Linear Model (GLM) was run, as well as the Mixed Linear Model (MLM; Yu et al., 2006) using three subpopulations (Q matrix) and the K matrix. To correct for multiple comparisons, an adjusted Bonferroni-corrected threshold (i.e., P [?] 1/N, where N is total number of genome-wide SNPs) was used for declaring the significance of GWAS associations (Benjamini and Hochberg 1995).

The Pathway Association Studies Tool (PAST; Thrash et al., 2020a) was used to perform a metabolic pathway analysis, as was first described in Tang et al. (2015). Linkage disequilibrium values between each marker and the 50 closest up- and downstream SNPs, and the SNP-trait association values for significance (p), correlation (R^2 or the proportion of the phenotypic variation accounted for), and allele effect as calculated by TASSEL were used by the PAST program to calculate the running enrichment score for all annotated maize pathways. A running enrichment score measures the probability that each pathway is associated with FAW resistance. The PAST program assigned each SNP to an annotated gene, based on user defined linkage

disequilibrium and physical distance values of$r^2 > 0.8$ and + 1 Kb, respectively. Each gene was assigned to a pathway using the gene annotation files in MaizeCyc (Monaco et al. 2013). Only pathways with five or more mapped genes (298 pathways) were considered in the analysis. More detail of the pathway analysis can be found in Warburton et al., (2017) and Li et al., (2019).

Targeted metabolite confirmation analysis

To test differential production of metabolites identified via PAST, leaves of the 31 most resistant lines in the study and 28 of the most susceptible lines based on the 7-day visual ratings from 2019 and 2020 were collected in 2021. Best and worst lines are indicated in Supplemental Table 1 in green or red highlight, respectively. Leaf tissue was collected from 10 plants of two replications of each genotype after the 14-day damage rating scores had been collected. Leaves from each plot of each selected genotype (except Mp704 and Mp707, which had too few live plants in the field) were harvested and flash frozen in liquid nitrogen and then used in a confirmation study whereby carotenoids and chlorophylls were extracted and analyzed for preferential abundance between the selected lines. This was done as previously described (Wojdyło et al., 2021) with the modification that magnesium carbonate (MgCO3, 50 mg) was added to lyophilized leaf samples (500 mg) to prevent isomerization. The samples were then extracted with 5 ml hexane:acetone:methanol (2:1:1, v/v/v) containing 1% BHT for 1 h in the dark on an orbital shaker (5000 rpm). The extracts were filtered through cotton plugs and the filtrates were concentrated via speedvac (Savant, Thermo Scientific). Each extract was re-dissolved in methanol (1 ml) and particulates were removed via centrifuge.

For carotenoid and chlorophylls analysis, the re-dissolved, centrifuged extracts were analyzed on a Waters Acquity UPLC system coupled to a Waters Xevo G2 XS Quadrupole Time-of-Flight (QTOF) mass spectrometer (MS). The QTOF MS was equipped with a Z-spray ionization source running in ESI+ mode using MassLynx 4.2 software with the following settings: source temperature: 100 °C, desolvation temperature: 250 °C, desolvation gas flow: 600 L/h, cone gas flow: 50 L/h, capillary voltage: 3.0 kV, sampling cone voltage: 40 V. Analyses were performed in sensitivity and continuum mode, with a mass range of m/z 50–1200 and a scan time of 0.1 s. A data-independent acquisition method with elevated collision energy (MSE) was used with 6 eV low energy and a high energy ramp from 15-45 eV. Separation was performed on a Waters BEH C18 1.7 μm, 2.1 x 50 mm column with the following gradient solvent system: (0.5 ml/min, solvent A: 0.1% formic acid in water; solvent B: 7:3 acetonitrile:methanol): 75% B (0.0-0.6 min.), gradient to 95.1% B (0.6-6.5 min.), gradient to 100% B (6.5-13.6 min.), 100% B (13.6-14.6 min.), then column equilibration to 5% B (14.6-17.5 min.). Data were analyzed on Waters UNIFI 1.9.4 software using the "Quantify Assay Tof 2D" analysis method with lock mass (leucine-enkephalin) corrected by UNIFI. Analytical standards (lutein and zeaxanthin) were purchased from ChromaDex and used to confirm identity of analytes and generate standard curves. Lutein and zeaxanthin are reported in μg carotenoid/g leaf sample. Relative amounts of chlorophyll a, chlorophyll b, and pheophytin b are reported as intensity counts/g leaf sample.

Normality of distribution on the metabolite levels was tested using QQplots and by performing a Shapiro-wilk test in R (Team, 2015). To achieve normality, metabolite content levels were transformed using log base 10. Normalized metabolite data and FAW ratings for day 7 and 14 from the 2021 field season were the input variables used to perform a correlation analysis using hierarchical clustering with 0.095 confidence intervals and 0.05 cut-off p-value in R. The data was scaled then used to generate a dendrogram by applying the Euclidean distance method and clustered by using the complete agglomeration method in R. Principal component analysis was performed using the same input variables used for the dendrogram. The first two PCs were used to create a PCA plot and variance by variable and individual was extracted from the analysis. Transformed and scale variables were plotted in relation to the cluster identity.

Results and Discussion

Variation for FAW damage in the GWAS panel

There was a wide range of variation in the damage ratings on the 289 inbred lines in the study, from a low of 2.01 to a high of 7.65 over the three years (Supplemental Table 1). The data over the three years of the study were well correlated, with correlations of 0.68 (2019 vs. 2021), 0.70 (2020 vs. 2021) and 0.72 (2019

vs. 2020). The 7-day ratings were also correlated with the 14-day ratings (0.94); (data not shown). The 31 most resistant inbred lines are highlighted in Supplemental Table 1 in green and include many lines from the USDA ARS CHPRRU (19 breeding lines and released inbred lines designated Mp); CIMMYT (10 released inbreds designated CML); and NCSU (1 inbred designated NC). Considering the large effect QTL identified in some of the CPHRRU lines in previous mapping studies (Brooks et al., 2007; Womack et al., 2018; 2020), and the strong resistance demonstrated by these lines in this study (all had average damage ratings of less than 4.0 over the three years), these lines would make excellent donor lines for introgression breeding of FAW resistance into elite but susceptible maize lines. The range of phenotypic variation was also sufficient for a successful GWAS study.

Genes associated with FAW damage ratings

When the GLM was used for association analysis, the observed -Log10(p values) were higher than the expected, as can be seen in the QQ plot in Supplemental Fig. 1a. The observed values were much closer to the expected when the MLM was used (Supplemental Fig. 1b), and thus the MLM results are presented here and were used in the subsequent pathway analysis. Because of missing data of some SNPs, several of the most significant SNPs ended up comparing only one or two individuals with one allele against one or more hundred with the other allele. To avoid this statistically risky analysis, the Minor Allele Frequency was increased to 0.09 and the GWAS run again with 940,585 remaining SNPs. Only results of this second analysis are presented here and were used in the pathway analysis. Setting a significance threshold of 1/n, there were 3 SNPs, corresponding to 2 linked loci, significantly associated at $p < 1.06 \times 10^{-6}$ with the data for FAW damage at 7 days after infestation, and 12 SNPs, corresponding to 5 linked loci, with the data at 14 days (Table 1). There was very good correspondence with the SNPs found to be associated in the 7-day and 14-day ratings. All 3 significantly associated SNPs in the 7-day rating analysis were associated in the 14-day analysis at the $p < 10^{-5}$ or better, and all but one of the 12 SNPs significantly associated in the 14-day rating analysis were associated in the 7-day analysis at the $p < 10^{-5}$ or better. The table of MLM association scores for $p < 0.05$ can be found in Supplemental Table 3. Associations that are better than the Bonferroni corrected threshold are highlighted in yellow.

Table 1: The 15 SNPs (listed by chromosome (Chr) and position along the chromosome) significantly ($p < 1.06 \times 10^{-6}$) associated with Fall Armyworm (FAW) damage levels measured at 7 and at 14 days after infestation were in or closely linked to 7 genes. Association values (degrees of freedom (df), F values, p values, and Error degrees of freedom (Error df) as well as correlations (Marker R^2), gene identifications ("Linked gene") and annotations ("Gene name or function") are noted.

7 Day FAW damage rating	**7 Day FAW damage rating**	**7 Day FAW damage rating**	**7 Day FAW dam**
Chr	**Position**	**df**	**F**
1	46380400	2	16.277
6	85713720	1	29.505
6	85715213	2	14.574
14 Day FAW damage rating	**14 Day FAW damage rating**	**14 Day FAW damage rating**	**14 Day FAW dar**
Chr	**Position**	**df**	**F**
4	15980038	2	18.029
4	15980032	2	18.029
1	177103292	2	16.568
8	22544824	2	16.246
3	139450676	1	31.336
3	139450701	1	31.336
3	139450683	1	31.336
3	139450697	1	31.336
3	139450106	1	25.460
3	139450107	1	25.460
3	139450108	1	25.460

7 Day FAW damage rating	7 Day FAW damage rating	7 Day FAW damage rating	7 Day FAW dam
5	191019633	2	14.932

The 15 SNPs associated with FAW damage levels were in or closely linked to 7 genes (Table 1), two of which were not annotated. The other five included a cyclin with kinase activity to promote cell division (cyc3, Zm00001d036360); a protein disulfide isomerase member of the thioredoxin superfamily involved in starch synthesis (pdi1, Zm00001d049099); a late embryogenesis abundant (LEA) hydroxyproline-rich member of the glycoprotein family that is associated with the plasma membrane (Zm00001d008850); a protein kinase domain containing protein located in the nucleus (Zm00001d041826); and a cell wall-associated receptor kinase-like 8 (Zm00001d017264). Three of these genes (Zm00001d028785, Zm00001d041826, and Zm00001d049099) were previously found to increase expression following exposure to fungal pathogens (Hoopes et al., 2019 and Swart et al., 2017). Although none of the genes were part of annotated maize pathways, one (Zm00001d049099) is a thioredoxin, and the thioredoxin pathway was found to be associated with FAW damage levels (see below).

Pathways associated with FAW damage levels

The pathway analysis was run in four separate tests on FAW damage. Pathways associated with reduced FAW damage scores at the $p < 0.05$ level were identified in the 7-day and 14-day ratings datasets, and pathways associated with increased FAW damage scores were run on the ratings datasets collected at 7 and 14 days. Following these pathway analyses, 31 pathways were associated with increased FAW damage: 26 with the 7-day ratings, 18 with the 14-day ratings, and 5 in common. Another 42 pathways were associated with decreased FAW damage: 29 with the 7-day ratings, 22 with the 14-day ratings, and 9 in common (Supplemental Table 4). There were no common pathways between the analyses for increased damage vs. decreased damage, but three related pathways were found between the increased and decreased analyses. Triacylglycerol biosynthesis was associated with reduced FAW damage, while triacylglycerol degradation was associated with increased damage; phospholipid biosynthesis was associated with increased damage, and phospholipid conversion to another compound was associated with reduced damage. On the other hand, UDP-β-L-arabinose biosynthesis was associated with both increased and decreased damage (via different pathways).

Some pathways share genes, and one or both pathways may become significantly associated with reductions in FAW damage scores. Which causes the reduction (or if both do) may not be readily apparent. For example, the top pathway for damage scores collected on both 7 and 14 days is the sporopollenin precursors biosynthesis pathway (32 genes total), at $p < 0.001$ and 0.006, respectively, but the suberin biosynthesis pathway (55 genes total) is also significantly associated with a reduction in FAW damage rated at 14 days, at $p < 0.05$, and the two pathways have 26 genes in common. The cutin biosynthesis pathway (42 genes) is significantly associated with reduced damage at 7 days, and cutin and sporopollenin precursors biosynthesis pathways also have 26 genes in common. However, suberin biosynthesis is not associated with reduced damage at 7 days and cutin is not associated with reduced damage at 14 days. All three pathways could plausibly be associated, but sporopollenin precursors (which are carotenoids and carotenoid esters) may have better statistical evidence for association. Most pathways with shared genes create related compounds, such as homoserine and L-homoserine biosynthesis. Most pathways contained several genes that jointly contributed to association with the trait. Few pathways had only one gene contributing to the trait and these, such as acyl-CoA hydrolysis, generally had only a few genes in the pathway so statistical evidence that they are actually associated is lower.

Looking at the 31 pathways associated at a more stringent significance threshold $p < 0.02$ (Table 2), mechanisms that may be highlighted by the current study as involved with FAW feeding resistance include the biosynthesis of carotenes including zeaxanthin and other pathways that also utilize geranylgeranyl pyrophosphate, including ent-kaurene and chlorophyll (Fig. 1). These are highly associated with FAW damage levels, both increasing and decreasing. Other mechanisms of resistance may include hormone signaling, as the IAA and sterol biosynthesis (of which brassinosteroids are a subset) as well as the ent-kaurene biosynthesis

pathway (which can be converted to gibberellins, Fig. 1) were all identified as associated with damage levels. Reactions associated with FAW damage (Table 2) include the S-adenosyl-L-methionine cycle, which was associated with increased damage; acyl-CoA hydrolysis (decreasing damage); and thioredoxins and glutathione redox reaction (related reactions, associated with decreased damage). The production of lipids and waxes, including cuticular wax, phosphatidylethanolamine, and phospholipids (compounds in biological membranes) and the essential oil linalool and β-caryophyllene), all associate with increased levels of damage. The biosynthesis of several other compounds is associated with decreased FAW feeding damage, including ascorbate, flavin or riboflavin, myo-inositol and phytate, and the amino acid cysteine. Some compounds associated with an increase in damage ratings include ammonia, 1,4-dihydroxy-2-naphthoate, the phenolic compound coumarin, and the amino acids homoserine, methionine, and homocysteine.

Figure 1 : Many of the pathways associated with Fall Armyworm leaf damage rating scores were in the carotenoid biosynthesis pathway or pathways branching off from or downstream of it. Pathways significantly associated with damage scores are circled in red (solid line circles $p < 0.02$; dashed line $p < 0.05$).

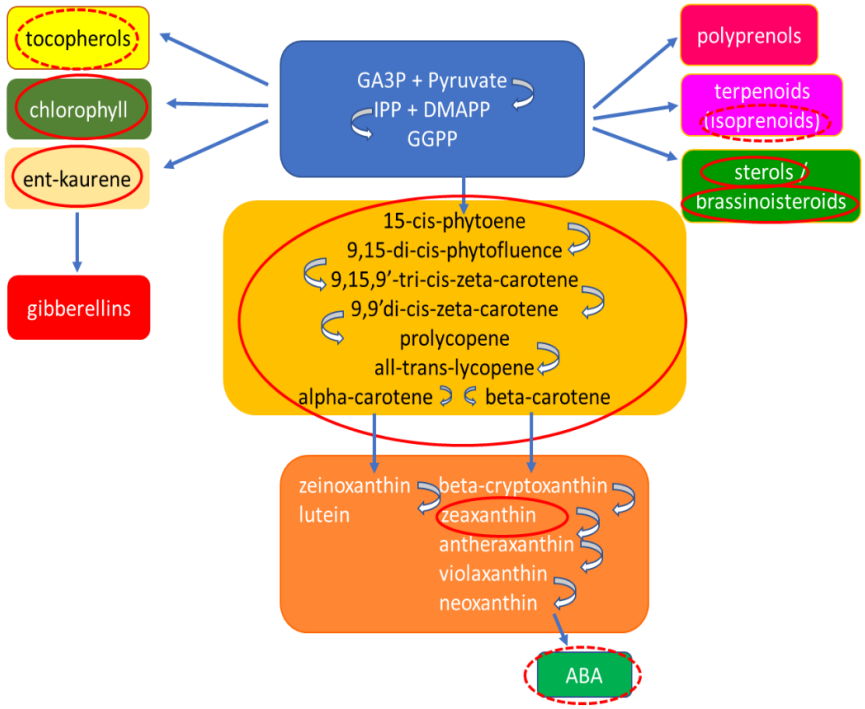


Table 2: The 31 pathways associated ($p < 0.02$) with Fall Armyworm leaf damage rating scores at 7 and 14 days after infestation, including pathways that increased and that decreased resistance in maize plants. Pathway identification (Pathway ID) from MaizeCyc, the pathway name, and association values (p and q value), the number of genes in the pathway (# genes) and notes about each pathway are included in the table.

Decreasing Damage at 14 days after infestation	**Decreasing Damage at 14 days after infestation**	
Pathway ID	**Pathway name**	**p value**
PWY-6733	sporopollenin precursors biosynthesis	0.0064
R-ZMA-1119348.1	Ent-kaurene biosynthesis	0.0066
R-ZMA-1119516.1	Trehalose biosynthesis I	0.0107
PWY-5148	acyl-CoA hydrolysis	0.0180

Decreasing Damage at 14 days after infestation	**Decreasing Damage at 14 days after infestation**	
Increasing Damage at 14 days after infestation	**Increasing Damage at 14 days after infestation**	
Pathway ID	**Pathway name**	**p value**
CAROTENOID-PWY	superpathway of carotenoid biosynthesis	0.0017
PWY-5791	1,4-dihydroxy-2-naphthoate biosynthesis II	0.0049
PWY-5944	zeaxanthin biosynthesis	0.0078
CHLOROPHYLL-SYN	3,8-divinyl-chlorophyllide a biosynthesis I	0.0108
PWY-282	cuticular wax biosynthesis	0.0118
PWY-5698	allantoin degradation to ureidoglycolate II	0.0137
HOMOSERSYN-PWY	L-homoserine biosynthesis	0.0177
R-ZMA-1119374.1	Abscisic acid biosynthesis	0.0469
Decreasing Damage at 7 days after infestation	**Decreasing Damage at 7 days after infestation**	
Pathway ID	**Pathway name**	**p value**
PWY-6733	sporopollenin precursors biosynthesis	0.0012
PWY3DJ-35471	L-ascorbate biosynthesis (partial pathway)	0.0013
CYSTSYN-PWY	L-cysteine biosynthesis I	0.0068
R-ZMA-1119370.1	Sterol biosynthesis	0.0097
HISTSYN-PWY	L-histidine biosynthesis	0.0109
R-ZMA-1119379.1	Flavin biosynthesis	0.0126
R-ZMA-1119378.1	Myo-inositol biosynthesis	0.0128
R-ZMA-1119434.1	Lipid-independent phytate biosynthesis	0.0128
THIOREDOX-PWY	Thioredoxin pathway	0.0147
PWY-2301	Myo-inositol biosynthesis	0.0150
R-ZMA-1119509.1	Histidine biosynthesis I	0.0160
R-ZMA-1119437.1	Glutathione redox reactions I	0.0182
Increasing Damage at 7 days after infestation	**Increasing Damage at 7 days after infestation**	
Pathway ID	**Pathway name**	**p value**
PWY-5041	S-adenosyl-L-methionine cycle II	0.0033
R-ZMA-1119486.1	IAA biosynthesis I	0.0075
R-ZMA-1119501.1	S-adenosyl-L-methionine cycle	0.0103
PWY-5669	phosphatidylethanolamine biosynthesis I	0.0130
PWY-6275	β-caryophyllene biosynthesis	0.0140
PWY-5176	coumarin biosynthesis (via 2-coumarate)	0.0162
PHOSLIPSYN2-PWY	superpathway of phospholipid biosynthesis II	0.0164
METHIONINE-DEG1-PWY	L-methionine degradation I (to L-homocysteine)	0.0172
PWY-6927	Chlorophyll *a* degradation II	0.0177

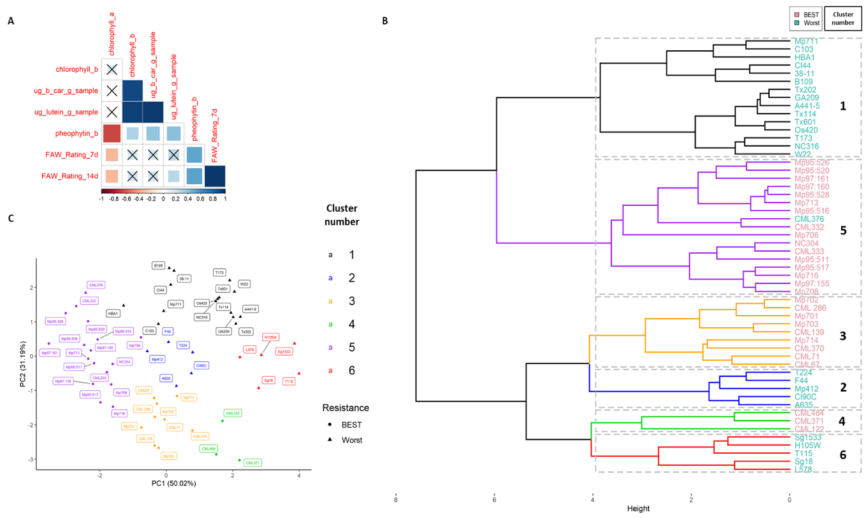
Validation of metabolites in resistant vs. susceptible maize lines

Using 29 of the most resistant and 28 of the most susceptible maize lines in this study, we sought correlations between the presence of zeaxanthin, ß-carotene, lutein, chlorophyll a, chlorophyll b, and pheophytin b, with 7- and 14-day damage rating. The carotenoids were measured because of the multiple carotenoid-related pathways identified by PAST, as were the chlorophylls and pheophytins. Pheophytins are identical in structure to chlorophylls but lack magnesium(II). Chlorophylls can be degraded to pheophytins under low pH or enzymatically. Along with chlorophylls and ß-carotene pheophytins are also involved in photosynthesis (photosystem II). We were unable to find measurable levels of zeaxanthin in the leaf samples, which is unsurprising as this compound tends to be produced in maize grains. How the biosynthesis pathway is then associated with reduced leaf feeding by FAW is thus unknown. The four other metabolites measured showed phenotypic variation among the maize lines tested, although about 80% of the lines showed low levels, with less than 300 µg/g of lutein, 2,000 µg/g of ß-carotene, 3E6 counts/g of chlorophyll a, 2E6 counts/g of chlorophyll b and 2E6 counts/g of pheophytin b (Supplemental Table 5).

Levels of these four metabolites were correlated with each other, and some with damage ratings (Fig. 2A). Lutein and pheophytin b were correlated with an increase in FAW damage, and chlorophyll a was correlated with a decrease in FAW damage, in agreement with pathway PWY-6927 (chlorophyll a degradation II) being associated with increased FAW damage ratings at 7 days (and less significantly so at 14 days, along with pathway PWY-5098, chlorophyll a degradation I), (Supplemental Table 4). Chlorophyll b is positively correlated to ß-carotene, lutein and pheophytin but negatively correlated with chlorophyll a. The chlorophyll cycle converts a to b forms of chlorophyll via a step through the intermediary 3,8-divinyl-chlorophyllide a; thus, it makes sense that a and b are negatively correlated and also that pathway CHLOROPHYLL-SYN, which produces 3,8-divinyl-chlorophyllide a, is associated with increased damage in the PAST analysis. ß-carotene was positively correlated to pheophytin and lutein, which were correlated to FAW damage, but ß-carotene itself was not significantly correlated with FAW damage scores. These metabolites appear to agree with the metabolic pathway analysis that found carotenoids to be important in FAW resistance but are not conclusive.

The wide distribution of phenotype in relation to metabolite content made straightforward conclusions difficult. The cluster analysis (Fig. 2B) and PCA (Fig. 2C) of metabolites and FAW ratings showed six groups, formed based on FAW rankings and also metabolite content. For example, two of the clusters showed high levels of lutein content, one of which were FAW-resistant and the other FAW-susceptible. For a clearer breakdown of metabolite and resistance phenotype, Supplemental Fig. 2 shows the levels of each metabolite within each of the six clusters; it is apparent that some of the clusters are highly significantly different than the others for levels of that metabolite. Although the numbers of lines within each cluster reduce the statistical power of the analysis, it is likely that within the resistant lines, there is more than one mechanism leading to resistance, but that levels of lutein, pheophytin, and chlorophyll a in particular are almost certainly contributing to resistance (or susceptibility). Although the effect of each metabolite on resistance may not be enough to create resistant lines, they do help to validate the results of the PAST metabolic pathway analysis.

Figure 2 : Targeted metabolite analysis for lutein, β-carotene, chlorophyll a and b and pheophytin b. A) Correlation matrix, B) dendrogram, and C) principal component analysis (PCA) of metabolite and Fall Armyworm rating analysis. In the dendrogram, maize lines are colored salmon (BEST) and blue (WORST) representing resistance phenotype. In the dendrogram and PCA, clusters are represented with the following colors, 1 = black, 2 = blue, 3 = yellow, 4 = green, 5 = purple and 6 = red. In the correlation matrix graph, blue represents positive correlation, red negative correlation and black "X" indicates that the comparison was not significantly correlated with a p-value cut-off of 0.05. In the PCA, shape of points represents resistant phenotype, circle (BEST) and triangle (WORST).



Pathways and genes in common with other larval insect pest resistance studies

A similar study was done previously by this group on corn earworm (*Helicoverpa zea* (Boddie)) ear-damage levels in maize (Warburton et al., 2017), and five of the same or very closely related pathways were identified in both studies. These include wax esters biosynthesis II, simple coumarins biosynthesis, geranylgeranyl diphosphate (GGPP) biosynthesis, the chlorophyll degradation pathway, ent-kaurene biosynthesis I, and phospholipases biosynthesis. Wax esters are a component of epicuticular wax, which is a physical barrier to insect predation. Coumarins belong to the phenolics class of compounds, which repel feeding insects. The production of GGPP is the first step leading into the carotenoids and related pathways (Fig. 1). Phospholipases may be expressed when plants are wounded, as during insect feeding, and initiate production of important defense signaling molecules, such as oxylipins and jasmonates (De Vleesschauwer et al., 2014). All these mechanisms were important in FAW resistance as well and may form part of the common defense mechanisms of maize plants against many larval feeding insects.

Another recent study of the defense response of resistant maize lines to European corn borer (*Ostrinia nubilalis*) and western corn rootworm (*Diabrotica virgifera virgifera*), two insects whose larvae are economically damaging. These two studies used transcriptomics to identify changes in gene expression following feeding damage (Pingault et al., 2021). They found significant gene expression changes in a number of genes, some in common with the current study. These include genes encoding, regulating, or modifying carotenoids, coumarins, S-adenosyl-L-methionine, methionine, Acyl-CoA, glutathione, thioredoxin, histidine, myo-inositol, flavin, sterol, trehalose, chlorophyll, phospholipases, and phospholipids. While this list is too long and varied to point to a specific common mechanism of resistance, it does suggest that common mechanisms may exist, and may indicate where the search may begin.

Acknowledgments

The Authors would like to thank Susan Wolf, Gerald Matthews and Carol Carter-Wientjes for excellent technical and editorial help. Any mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

Conflict of Interest

The authors declare no conflict of interest.

ORCID

Marilyn L. Warburton: https://orcid.org/0000-0002-9542-9912

Lina Castano-Duque: https://orcid.org/0000-0001-9161-2907

W. Paul Williams: https://orcid.org/0000-0002-7827-3186

Matthew D. Lebar: https://orcid.org/0000-0003-4910-1438

Sandra W. Woolfolk: https://orcid.org/0000-0001-7025-9745

Supplemental Material

Supplemental Figure 1: QQ plot of association values calculated with the General Linear Model (GLM; 1a) and Mixed Linear Model (MLM; 1b).

Supplemental Figure 2: Box plots showing clusters and the distribution of the amounts of metabolites and Fall Armyworm ratings. Box-plot whiskers depict the maximum and minimum without outliers, and the box depicts median, first and third quantiles distribution. Data shown is transformed and scale to center around 0.

Supplemental Table 1: Fall Armyworm damage scores at 7 and 14 days after infestation for the panel of 289 diverse maize inbred lines used for this study, including averages and standard deviations over replications within years, and averaged over years.

Supplemental Table 2: Fall Armyworm damage score descriptions, from the original rating scale of Davis et al., 1991, for 7-day and 14-day time points following infestation with Fall Armyworm (FAW) neonates.

Supplemental Table 3: The MLM association scores for $p < 0.05$ associated with Fall Armyworm (FAW) damage levels.

Supplemental Table 4 : The pathways associated ($p<0.05$) with Fall Armyworm (FAW) damage levels.

Supplemental Table 5: Averaged data for the metabolites analyzed, clusters summary statistics and PCA variance contribution information.

References

Bradbury, P.J., Zhang, Z., Kroon, D.E., Casstevens, T.M., Ramdoss, Y., & Buckler, E.S. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics* , *23* (19), 2633-2635. https://doi.org/10.1093/bioinformatics/btm308

Benjamini Y. & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing.*Journal of the Royal Statistical Society: Series B (Methodological)* , *57* (1), 289-300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Brooks, T.D., Bushman, B.S., Williams, W.P., McMullen, M.D., & Buckley, P.M. (2007). Genetic basis of resistance to and southwestern corn borer (Lepidoptera: Crambidae) leaf-feeding damage in maize. *Journal of Economic Entomology* , *100* (4), 1470-1475. https://doi.org/10.1093/jee/100.4.1470

Davis, F.M. (1989). Rearing the southwestern corn borer and fall armyworm at Mississippi State. In, *Toward insect resistance maize for the third world: Proceedings of the International Symposium on Methodologies for Developing Host Plant Resistance to Maize Insects .CIMMYT, Mexico, March 9-14, 1987* . (pp. 27-36). CIMMYT.

Davis, F.M., Ng, S.S., & Williams, W.P. (1992). *Visual rating scales for screening whorl-stage corn for resistance to fall armyworm*(Technical Bulletin 186). Mississippi Agricultural and Forestry Experiment Station.

Davis, F.M., Williams, W.P., & Buckley, P.M. (1998). Growth responses of southwestern corn borer (Lepidoptera: Crambidae) and fall armyworm (Lepidoptera: Noctuidae) larvae fed combinations of whorl leaf tissue from a resistant and a susceptible maize hybrid. *Journal of Economic Entomology* , *91* (5), 1213-1218. https://doi.org/10.1093/jee/91.5.1213

Davis, F. M., Williams, W. P., Chang, Y. M., Baker, G. T., & Hedin, P. A. (1999). Differential growth of fall armyworm larvae (Lepidoptera: Noctuidae) reared on three phenotypic regions of whorl leaves from a resistant and a susceptible maize hybrid. *Florida Entomologist* ,*82* (2), 248-254.

De Vleesschauwer, D., Xu, J. & Höfte, M. (2014). Making sense of hormone-mediated defense networking: from rice to Arabidopsis.*Frontiers in Plant Science* , *5* , 611. https://doi.org/10.3389/fpls.2014.00611

Flint-Garcia, S.A., Thuillet, A.C., Yu, J.M., Pressoir, G., Romero, S.M., Mitchell, S.E., Doebley, J., Kresovich, S., Goodman, M.M., & Buckler, E.S. (2005). Maize association population: a high resolution platform for quantitative trait locus dissection. *The Plant Journal, 44* (6), 1054-1064. https://doi.org/10.1111/j.1365-313X.2005.02591.x

Glaubitz, J.C., Casstevens, T.M., Lu, F., Harriman, J., Elshire, R.J., Sun, Q. & Buckler, E.S. (2014). TASSEL-GBS: a high capacity genotyping by sequencing analysis pipeline. *PloS One* , *9* (2), e90346. https://doi.org/10.1371/journal.pone.0090346

Goergen, G., Kumar, P.L., Sankung, S.B., Togola, A., & Tamò, M.. (2016). First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae), A new alien invasive pest in West and Central Africa. *PLoS One* , *11* (10), e0165632. https://doi.org/10.1371/journal.pone.0165632

Hoopes, G.M., Hamilton, J.P., Wood, J.C., Esteban, E., Pasha, A., Vaillancourt, B., Provart, N.J., & Buell, C.R. (2019). An updated gene atlas for maize reveals organ-specific and stress-induced genes. *The Plant Journal* , *97* (6), 1154-1167. https://doi.org/10.1111/tpj.14184

Li, H., Thrash, A., Tang, J.D., He, L., Yan, J., & Warburton, M.L. (2019). Leveraging GWAS data to identify metabolic pathways and networks involved in maize lipid biosynthesis. *The Plant Journal* ,*98* (5), 853-863. https://doi.org/10.1111/tpj.14282

McMullen, M.D., Frey, M., & Degenhardt, J. (2009). Genetics and biochemistry of insect resistance in maize. In J.L. Bennetzen & S.C. Hake (Eds.), *Handbook of maize: Its biology* (pp. 271-289). Springer.

Mihm, J. A., Smith, M.E., and Deutsch, J.A. (1988). Development of open-pollinated varieties, non-conventional hybrids and inbred lines of tropical maize with resistance to fall armyworm, *Spodoptera frugiperda (* Lepidoptera: Noctuidae), at CIMMYT. *Florida Entomologist* , *71* (3), 262-268.

Monaco, M.K., Sen, T.Z., Dharmawardhana, P.D., Ren, L., Schaeffer, M., Naithani, S., Amarasinghe, V., Thomason, J., Harper, L., Gardiner, J., Cannon, E.K.S., Lawrence, C.J., Ware, D., & Jaiswal, P. (2013). Maize metabolic network construction and transcriptome analysis. *The Plant Genome* , *6* (1), plantgenome2012.09.0025. https://doi.org/10.3835/plantgenome2012.09.0025

Overton, K., Maino, J.L., Day, R., Umina, P.A., Bett, B., Carnovale, D., Ekesi, S., Meagher, R., & Reynolds, O.L. (2021). Global crop impacts, yield losses and action thresholds for fall armyworm. (Spodoptera frugiperda): A review. *Crop Protection* , *145* , 105641 https://doi.org/10.1016/j.cropro.2021.105641

Pingault, L., Basu, S., Zogli, P., Williams, W.P., Palmer, N., Sarath, G., & Louis, J. (2021). Aboveground Herbivory Influences Belowground Defense Responses in Maize. *Frontiers in Ecology and Evolution* ,*9* , 765940. https://doi.org/10.3389/fevo.2021.765940.

Scott, G.E., & Davis, F.M. (1981). Registration of Mp496 inbred of maize. *Crop Science* , *21* (2), 353. https://doi.org/10.2135/cropsci1981.0011183X002100020049x

Scott, G. E., Davis, F.M., and Williams, W.P. (1982). Registration of Mp701 and Mp702 germplasm lines of maize. *Crop Science* ,*22* (6), 1270. https://doi.org/10.2135/cropsci1982.0011183X002200060070x

Swart, V., Crampton, B.G., Ridenour, J.B., Bluhm, B.H., Olivier, N.A., Meyer, J.J.M., & Berger, D.K. (2017). Complementation of CTB7 in the maize pathogen *Cercospora zeina* overcomes the lack of in vitro cercosporin production. *Molecular Plant Microbe Interactions* ,*30* (9), 710-724. https://doi.org/10.1094/MPMI-03-17-0054-R

Tang, J.D., Perkins, A., Williams, W.P., and Warburton, M.L. (2015). Using genome-wide associations to identify metabolic pathways involved in maize aflatoxin accumulation resistance. *BMC Genomics* ,*16* , 673. https://doi.org/10.1186/s12864-015-1874-9

Team, R.C. (2015). R: a language and environment for statistical computing [Online]. Vienna, Austria: R Foundation for Statistical Computing. Available: https://www.R-project.org.

Thrash, A., Tang, J.D., DeOrnellis, M., Peterson, D.G., & Warburton, M.L. (2020a). PAST: The Pathway Association Studies Tool to infer biological meaning from GWAS datasets. *Plants 9* (1), 58. https://doi.org/10.3390/plants9010058

Thrash, A. & Warburton, M.L. (2020b). A Pathway Association Study Tool for GWAS analyses of metabolic pathway information . *Journal of Visualized Experiments* , *161* , e61268. https://doi.org/10.3791/61268-v

Turnbull, C., Lillemo, M., & Hvoslef-Eide, T.A. (2021). Global regulation of genetically modified crops amid the gene edited crop boom - A review. *Frontiers in Plant Science* , *12* , 630396. https://doi.org/10.3389/fpls.2021.630396

Warburton, M.L., Williams, W.P., Windham, G., Murray, S., Xu, W., Hawkins, L., & Franco, J. (2013). Phenotypic and genetic characterization of a maize association mapping panel developed for the identification of new sources of resistance to *Aspergillus flavus*and aflatoxin accumulation. *Crop Science* , *53* (6), 2374-2383. https://doi.org/10.2135/cropsci2012.10.0616

Warburton, M.L, Womack, E., Tang, J.D., Thrash, A., Smith, J.S., Xu, W., Murray, S.C., & Williams, W.P. (2017). Genome-wide association and metabolic pathway analysis of corn earworm resistance in maize.*The Plant Genome* , *11* (1), 170069. https://doi.org/10.3835/plantgenome2017.08.0069

Williams, W. P., & Davis, F.M. (1980). Registration of Mp703 germplasm line of maize. *Crop Science, 20* (3), 418. https://doi.org/10.2135/cropsci1980.0011183X002000030052x

Williams, W.P., & Davis, F.M. (1982). Registration of Mp704 germplasm line of maize. *Crop Science* , *22* (6), 1269-1270. https://doi.org/10.2135/cropsci1982.0011183X002200060068x

Williams, W.P., & Davis, F.M. (1984). Registration of Mp705, Mp706, and Mp707 germplasm lines of maize. *Crop Science* , *24* (6), 1217. https://doi.org/10.2135/cropsci1984.0011183X002400060062x

Williams, W.P., Davis, F.M., & Windham, G.L. (1990). Registration of Mp708 germplasm line of maize. *Crop Science* , *30* (3), 757. https://doi.org/10.2135/cropsci1990.0011183X003000030082x

Williams, W.P., Sagers, J.B., Hanten, J.A., Davis, F.M., & Buckley, P.M. (1997). Transgenic corn evaluated for resistance to fall armyworm and southwestern corn borer. *Crop Science* , *37* (3), 957-962. https://doi.org/10.2135/cropsci1997.0011183X003700030042x

Williams, W.P., Davis, F.M., Buckley, P.M., Hedin, P.A., Baker, G.T., & Luthe, D.S. (1998). Factors associated with resistance to fall armyworm (Lepidoptera: Noctuidae) and southwestern corn borer (Lepidoptera: Crambidae) in corn at different vegetative stages. *Journal of Economic Entomology* , *91* (6): 1471-1480. https://doi.org/10.1093/jee/91.6.1471

Williams, W.P., & Davis, F.M. (2000). Registration of maize germplasms Mp713 and Mp714. *Crop Science* , *40* (2), 584. https://doi.org/10.2135/cropsci2000.0015rgp

Williams, W.P., & Davis, F.M. (2002). Registration of maize germplasm line Mp716. *Crop Science* , *42* (2), 671-672. https://doi.org/10.2135/cropsci2002.671a

Wiseman, B.R., Davis, F.M., & Campbell, J.E. 1980. Mechanical infestation device used in fall armyworm plant resistance program.*Florida Entomologist* , *63* (4), 425-432.

Wojdyło, A., Nowicka, P., Tkacz, K., & Turkiewicz, I.P. Fruit tree leaves as unconventional and valuable source of chlorophyll and carotenoid compounds determined by liquid chromatography-photodiode-

quadrupole/time of flight-electrospray ionization-mass spectrometry (LC-PDA-qTof-ESI-MS), 2021, Food Chemistry, 349, 129156. https://doi.org/10.1016/j.foodchem.2021.129156

Womack, E.D., Williams, W.P., Smith, J.S., Warburton, M.L. & Bhattramakki, D. (2020). Mapping quantitative trait loci for resistance to fall armyworm (Lepidoptera: Noctuidae) leaf damage in maize inbred Mp705. *Journal of Economic Entomology* , *113* (2), 956-63. https://doi.org/10.1093/jee/toz357

Womack, E.D., Warburton, M.L., & Williams, W.P. (2018). Mapping of quantitative trait loci for resistance to fall armyworm and southwestern corn borer leaf-feeding damage in maize. *Crop Science* ,*58* (2), 529-539. https://doi.org/10.2135/cropsci2017.03.0155

Yu, J., Pressoir, G., Briggs, W.H., Bi, I.V., Yamasaki, M., Doebley, J.F., McMullen, M.D., Gaut, B.S., Nielsen, D.M., Holland, J.B., Kresovich, S., & Buckler, E.S. (2006). A unified mixed-model method for association mapping that accounts for multiple levels of relatedness.*Nature Genetics* , *38* (2), 203-208. https://doi.org/10.1038/ng1702