THESIS

METABOLOMIC PROFILES ASSOCIATED WITH PHYSIOLOGICAL RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY IN COMMON BEAN

Submitted by

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ABSTRACT

METABOLOMIC PROFILES ASSOCIATED WITH PHYSIOLOGICAL RESISTANCE TO *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY IN COMMON BEAN

Common bean (*Phaseolus vulgaris* L.) is an important global food crop with a recently sequenced and annotated genome. Plant metabolic and hormone processes are being increasingly recognized as central to disease resistance. For common bean, the molecular and metabolic processes that mediate resistance to white mold disease (caused by *Sclerotinia sclerotiorum*, (Lib.) de Bary) are largely unknown. Identifying metabolites associated with *Sclerotinia* infection may provide novel targets to breed for enhanced resistance. The metabolic changes that occur during *S. sclerotiorum* infection of a detached leaf were characterized using a non-targeted metabolomics workflow spanning primary and secondary metabolism, and a targeted panel of 13 hormones. Partial resistant (A195, beige seed coat color) and susceptible (Sacramento, light red kidney market class) Andean bean lines were inoculated with isolate S20 for non-targeted metabolite profiling at 16, 24, and 48 hours post inoculation (hpi) and at 8 and 16 hpi for hormones. Metabolites from healthy tissue adjacent to the necrotic lesion were extracted with the solvent methanol:water (80:20) and detected using non-targeted UPLC-TOF-MS and GC-MS workflows, and hormones were profiled using UPLC-MS/MS. The analysis detected 140 metabolites that varied between A195 and Sacramento, with the greatest metabolite variation occurring at 16 hpi. The metabolites that varied included amines, amino acids, saccharides, organic acids, phytoalexins, hormones, ureides, and molecules involved in cell wall and membrane composition. The diversity in observed metabolic changes points towards a

multi-faceted response associated with plant resistance to *S. sclerotiorum* in common bean. The integration of metabolomics and genomic data discover functional markers of metabolic resistance to white mold.

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CHAPTER ONE / INTRODUCTION

Common bean is an important global staple food crop

Common bean (bean, *Phaseolus vulgaris* L.) is one of the major staple food crops and an important source of nutrition in the United States and abroad. Among grain crops, "grain legumes" rank third in total world production behind cereals and oil seeds [1].

For developing countries, common bean is an important source of macro- and micronutrients and is critical to preventing severe malnutrition in areas with low food diversity in the diet. [2]. Beans are commonly described as "nutritionally rich" for their high content of protein, folic acid, and fiber.

Sclerotinia sclerotiorum is an important pathogen in common beans

Controlling plant disease is a challenge for common bean production and is a major threat to food security [2]. Compared to chemical control of pathogens, identifying genetic resistance is critical to be able to distribute germplasm to both large and small scale growers, and can be significantly more cost-effective and a more robust defense. Thus, it is important to better understand the genetic basis of resistance to pathogens to develop resistant cultivars as sustainable, accessible, and cost-effective measures to mediate disease.

White mold disease (WM), caused by the necrotrophic ascomycete fungal pathogen *Sclerotinia sclerotiorum,* is one of the most destructive soil borne pathogens and has been reported to infect over 400 species of plants resulting in crop losses exceeding $200 million dollars annually in the United States [3] [4] [5]. The disease is widespread, occurring across

major bean production regions in the United States, South America, Europe, Australia, and in some African and Asian countries [6]. In beans, this disease affects total yield by reducing seed size, and pod number, and up to 100% loss can occur under ideal environmental conditions for pathogen growth [7], [8] [9]. Thus, WM disease is a major problem for bean production in the United States [10].

WM disease is largely affected by environment. The bean production environment can result in a unique microclimates for *Sclerotinia* growth. During the R1-R3 growth stages, the bean canopy begins to close during flowering. This closure can provide a cool and moist microclimate which allows for *Sclerotinia* to infect flowers and form apothecia. The need for an optimal microclimate has both positive and negative attributes for managing WM disease; WM disease is sporadic (not an annual problem), but when the microclimate is optimal the disease is aggressive, and the closed canopy makes it difficult to diagnose and treat. [7]. [6].

S. sclerotiorum uses multiple mechanisms to infect, penetrate, colonize, and induce cell death in host plant tissues. Infection typically occurs when windblown ascospores infect senescent blossoms. After infection, the fungus develops mycelia that proceed to colonize the host plant (Fig. 1) [11], [8]. Ascospores use senescent blossoms as a nutrient source to allow for germination and induction of appressoria, structures that penetrate the leaf epidermis to facilitate colonization host leaves and stems [6]. Upon colonization of plant tissue, the pathogen forms sclerotia that can be deposited into the soil where they lay dormant until future seasons.

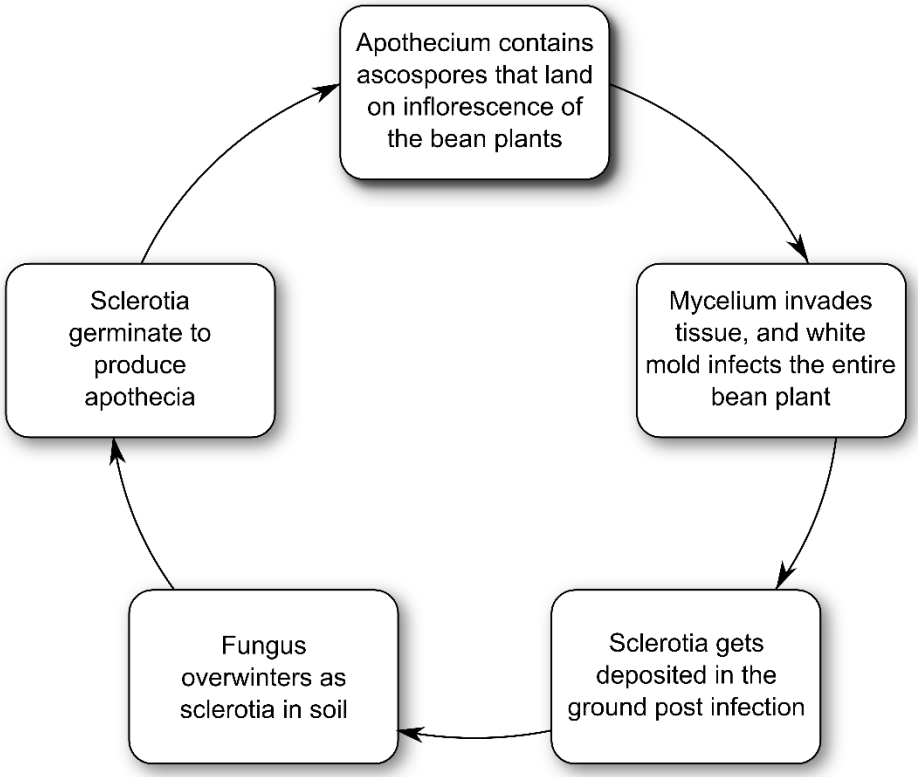

Figure 1: Life cycle of *Sclerotinia sclerotiorum* and disease symptoms in bean plants**.**

The importance of small molecules in the bean-Sclerotinia interaction warrants investigation to further our understanding of the molecular basis of bean resistance to Sclerotinia. During infection, *S. sclerotiorum* secretes oxalic acid onto plant tissue to allow for cell death and colonization. Oxalic acid is a dicarboxylic acid that is considered a nonspecific phytotoxin and contributes to reduction in pH, elicitation of programmed cell death (PCD), acidity-induced activation of pathogenic enzymes, guard cell regulation, and has a dual role (first suppressing and then stimulating) in regulating reactive oxygen species (ROS) [12]. The generation of ROS (e.g. hydrogen peroxide and superoxide) by the host plant is one of the earliest and most universal responses of plants to biotic stress. During the initial stages of infection, oxalic acid creates an environment with reduced pH in the host cells that suppress host defense responses. This is accomplished by inhibiting the plant oxidative burst and the deposition of callose. This is supported by studies that demonstrate that plants infected with oxalic acid deficient mutants accumulate higher amounts of hydrogen peroxide compared to plants infected with wild type (WT) fungi [5]. Once infection is established, oxalic acid acts as a signaling molecule that induces PCD through increased synthesis of ROS. At a more normal pH (> 5) and low levels of oxalic acid, ROS induce DNA fragmentation and PCD is triggered [12]. As oxalic acid accumulates, pH decreases to an optimal 4, allowing for activation of cell wall degrading enzymes (e.g. polygalacturonase) that result in necrosis [7] [10] [13]. Interestingly, common bean resistance to Sclerotinia is associated with tolerance to oxalic acid, independent of pathogen presence [7] [9]. Godoy et al. demonstrated that *Sclerotinia sclerotiorum* is nonpathogenic in the absence of oxalic acid [14].

Oxalic acid also interferes with guard cell function by stimulating the uptake of potassium ions and degradation of starch [13] [15], resulting in abnormal stomatal opening during infection [4], [5]. Research has shown that when broad bean (*Vicia faba* (L.) is infected with WT *S. sclerotiorum*, stomatal conductance and leaf transpiration rates increase, resulting in reduced biomass and foliar wilting due to lack of stomatal closure compared to oxalate deficient mutants [13]. *S. sclerotiorum* also interferes with abscisic acid (ABA) -induced stomatal closure [15]. It is suggested that ABA, through an antagonistic interaction with oxalic acid, contributes to resistance against *S. sclerotiorum* [13]. Infection induces stomata to open in advance of hyphae and provides an entrance for the hyphae to penetrate through stomata rather than through the cuticle.

Genetic resistance is critical control WM disease.

Cultural methods to control WM have had limited success in the field. Attempts have been made to reduce the spread of the fungus using crop rotation, increased plant spacing by reducing population density, residue management, timed irrigation, biological control, and timely application of fungicides [4], [16], [6]. These methods have provided only partial control due to the broad fungal host range and the durability of sclerotial bodies in the soil for many years.

Genetic mechanisms of defense to *Sclerotinia* utilize both physiological and avoidance traits. However, these traits fail to provide a durable form a resistance because they are significantly affected by environment, such as temperature and soil. Plant avoidance mechanisms include bean cultivars with early flowering, plant maturity, and upright

architecture. However, even with the development of avoidance traits, WM continues to be a major problem in the U.S.

One of the earliest physiological defenses following infection is the cellular oxidative burst which involves a rapid, transient production of large amounts of ROS that results in PCD [17]. Physiologically, PCD can be positive or negative effects on the development of plant disease. In the case of *S. sclerotiorum,* PCD accelerates WM disease because the components released following cell death are used as nutrients for Sclerotinia [4]. A second defense response is cell wall reinforcement via callose deposition [17]. Further, plant hormones that affect metabolism have been shown to be important to *Arabidopsis thaliana* defense against Sclerotinia including jasmonic acid, salicylic acid, and ethylene [5].

Metabolomics is a method that can characterize the interaction between bean and *S. sclerotiorum*.

The plant immune system is largely made up of small molecules that includes both primary and secondary metabolites. The class of phytochemicals that provide immunity is high diverse and include flavonoids, terpenoids, and organic acids. [18]. Plant metabolites can be evaluated using a metabolomics, the global analysis of small molecules, and is described as a key extension in plant functional genomics [19].

To understand mechanisms of plant defense in sunflower *(Helianthus* L), a metabolomics experiment was found that primary metabolites were important for defense against *S. sclerotiorum* [18]. In this study, increased abundance of metabolites in the resistant and

susceptible lines appears occur upstream of ethylene, salicylic acid, and polyphenolic compounds. Additionally, secondary metabolism is often connected to interactions with the environment and is thought to play a large role in the success of plant adaptation. Researchers found metabolites linked to photorespiratory regulation (e.g. glycine) to be upregulated in resistant plants post inoculation, suggesting a role in mediating defense mechanisms to *S. sclerotiorum*. Researchers also found contrasting evidence of nitrogen mobilization in plants in response to *S. sclerotiorum* [19]. For example, metabolites such as glutamate and asparagine had increased levels in the susceptible lines compared to resistant lines in response to infection.

Metabolomic Profiling can provide insight into genetic resistance to *S. sclerotiorum*.

Genetic resistance has been identified in several bean lines, although resistance has been described as highly quantitative. Quantitative trait loci (QTL) are regions of the plant genome that contain or are linked to genes that control plant quantitative traits. QTLs often have minor effects and interact with the environment [16], and thus have been difficult to integrate into commercially acceptable high yield cultivars [20]. In common bean, QTL conferring partial resistance to WM have been identified and mapped, however, the biochemical basis for resistance is largely unknown [10]. There is a need to investigate the biochemical mechanisms of common bean resistance to WM. Metabolomic profiling has the potential to provide insight into mechanisms of physiological resistance controlled by QTL in common bean.

The objectives of the following research were to (i) determine metabolite profiles associated with genetic, physiological resistance to Sclerotinia (ii) determine if phytohormones are important for common bean resistance and (iii) develop a model to describe metabolic pathways that provide resistance in common bean.

CHAPTER TWO / MATERIALS AND METHODS

Overview

White mold resistance in common bean can be attributed to both avoidance and physiological resistance mechanisms [16]. The mechanisms of resistance to white mold in common bean are often confounded in the field making it difficult to distinguish between the contribution of avoidance and physiological resistance. To eliminate the uncertainty of contribution of different mechanisms of resistance, a detached leaf assay was used to focus on molecular mechanisms associated with physiological resistance to *Sclerotinia sclerotiorum* in common bean.

Two bean lines that differ in response to *S. sclerotiorum* based on the Petzoldt and Dickson Straw Test (Petzoldt and Dickson, 1996) were selected for experimental analysis [21]. Leaves of the two lines were subjected to gas exchange analysis, leaf pH analysis and metabolic profiling to determine potential physiological resistance mechanisms. Leaves were inoculated using a detached leaf assay, after which, tissue samples were taken at time points 0, 16, 24, and 48 hours post inoculation (hpi) for metabolite extraction. Leaf extracts from resistant and susceptible lines were screened for metabolic variation following inoculation using a non-targeted and targeted metabolomic workflow. The relative abundance of each metabolite was compared to identify metabolites that differed in abundance between A195 and Sacramento. Differing metabolites were analyzed using gene set enrichment analysis to determine enriched metabolic pathways. The methods above allowed for both the discovery of novel metabolic

processes, as well as confirmed metabolites previously shown to be involved in disease resistance.

Plant material

Two bean lines that differ for resistance to *S. sclerotiorum* were chosen to evaluate the response to white mold infection. A195 and Sacramento were selected as the resistant and susceptible dry bean lines, respectively. Both beans lines are inbred (homozygous) and belong to the Andean South American common bean race Nueva Granada to reduce genetic variability [22]. Lines were maintained by the Dry Bean Breeding project of Colorado State University in Fort Collins and propagated in the greenhouse.

A195 is a resistant dry bean line developed from a single cross of 'Red Kloud'/ICA 1009 at the Centro Internacional de Agricultura Tropical (CIAT), Palmira, Colombia in 1979 [23]. A195 has an upright determinate Type I growth habit allowing for disease avoidance [23]. A195 seed was obtained from Dr. Shree P. Singh (University of Idaho, Moscow, ID). Sacramento (Sac) is a commercial large-seeded light red kidney dry bean cultivar [24] [25]. Sacramento was developed from a single plant selection for earliness from the cultivar 'California Light Red Kidney' and released by Sacramento Valley Milling Co. and the University of California, Davis in 1976. Sacramento also has an upright Type I growth habit allowing for disease avoidance [26], however, it is classified as susceptible when evaluated in physiological resistance assays. Sacramento seed was obtained from Dr. Ron Shellenberger, Provita, Inc., of Nampa, Idaho (stock 12-587). A195 was chosen as the resistant line for its relatively high level of resistance in

common bean [23]. Sacramento was chosen as the susceptible line because it has known high susceptibility to *S. sclerotiorum*.

Greenhouse growth conditions for all experiments

Untreated seed from A195 and Sacramento were planted in 4.5 L pots with pre-treated high porosity potting media. The media was pre-treated with 10 grams Osmocote™ 19-5-8 time release fertilizer with micronutrients. Root Shield™ (*Trichoderma harzianum*, strain T-22) was applied as a powder to the soil to control fungus gnats (*Lycoriella* spp. and *Bradysia* spp.). Three seeds were planted and thinned to an experimental unit of 2 plants per pot. Pots were watered to field capacity immediately after seeding. Plants were grown in a greenhouse with a 16/8 hr light/dark photoperiod. A timed drip irrigation system was used to water plants daily (~5 L/pot). No herbicides, pesticides or fungicides were used, and temperature and humidity were controlled by automated methods. Upper and lower heating and cooling set point were 20 °C and 26 °C during the day, and 17 °C and 24 °C at night. Relative humidity was maintained at 55% during the day and 75% at night. Plants were grown in the greenhouse to minimize the influence of environment on the results of the straw test or metabolite variation.

Inoculum for all studies

Three types of *Sclerotinia sclerotiorum* inocula were used including a virulent and avirulent type, and mock inoculation control (no Sclerotinia). All inocula plugs were cut as a ~7

mm diameter circles from the respective Petri plates, and applied by placing inocula (mycelia) in direct contact with leaf or stem tissue.

The virulent inoculum was cultured from sclerotia of the dry bean isolate Ss20 [27] provided by Dr. Howard F. Schwartz ,Colorado State University, Fort Collins, CO. Virulent inoculum was taken from the advancing edge of growing mycelia on Petri plates. The avirulent strain was cultured from mycelia transferred from filter paper of the oxalate deficient mutant dry bean isolate A4, provided by Dr. James R. Steadman, University of Nebraska, Lincoln, NE [28]. Virulent and avirulent inoculum was grown for 3-5 days on PDA in the dark at 22°C [3]. The mock inoculum was pure Potato Dextrose Agar (PDA) with no fungal presence.

Petzoldt and Dickson Straw Test

Resistance of A195 and Sacramento to WM were validated using the Petzoldt and Dickson Straw Test [21]. Straw Tests were conducted on eight greenhouse grown plants with two plants per plot. After plants reached the V3 growth stage, stems were cut 2.5 cm above the third internode for inoculation. Two mycelial plugs were cut from Petri plates of either virulent or mock inoculum, stacked into a pipette tip, and placed over freshly cut stems. The pipette tips (~7 mm diameter) provided the initial source of inoculum and were allowed to remain on the cut stems throughout the course of the experiment.

After inoculation, plants were immediately transferred to a chamber that misted for 30 sec every 5 min. Disease severities were evaluated 14 days post-inoculation on a 1 to 9 scale based on mycelial growth, where 1= no mycelial growth beyond the point of inoculation, 2=

mycelial growth half way towards the first node, 3= mycelial growth up to the first node, 4= mycelial growth into the first node, but not past the first node 5= mycelial growth past the first node, but not half way to the second node, 6= mycelial growth over half way to the second node, 7= mycelial growth into the second node, 8= mycelial growth past the second node, but still had green stem below infection, and 9= total plant death [29].

Gas exchange analysis

Gas exchange differences were evaluated in A195 and Sacramento in response to virulent and mock inoculum. To evaluate gas exchange differences, photosynthetic rate (Ps), stomatal conductance (Cs) and transpiration rate (Tr) were analyzed. Attached leaves in the upper trifoliate (V5 stage) were inoculated with virulent or mock inoculum. The inoculum plugs were placed face down adjacent to the mid-rib, approximately1/3 distal from the petiole. The inocula plugs were covered with a piece of square plastic wrap (~3 cm^2) to secure the plug and prevent dehydration. The plants were maintained in an open bench with the inoculum for 48 hrs, and then gas exchange analysis measurements were taken directly adjacent to the mock or virulent plugs. There were six replicates per treatment which consisted of leaves from three plants, with two leaves per plant.

Gas exchange characteristics were collected in sunlight at 9:30 am using the open gas exchange system LI-6400 photosynthesis system (LICOR, Lincoln, NE). Measurements were automatically repeated five times for all replicates. The LICOR had a lamp setting of 700, block temperature of ~25° C, mixer and flow of 400, and the leaf fan set at 3. The gas exchange

analysis was used to quantify photosynthetic rate, stomatal conductance and transpiration rate for A195 and Sacramento compared to their mock inoculum controls.

Detached leaf assays for pH analysis

For pH analysis, A195 and Sacramento were grown for approximately six weeks in a greenhouse. Leaves were harvested before plants reached reproductive stages. Fully expanded healthy leaves were detached at the petiole from the upper trifoliolate (approximately V5 stage) and placed into Petri dishes (100 mm x 15 mm) with a damp paper towel [30].

After leaves were detached and transferred to a Petri dish, an agar plug of inoculum or mock inoculum control (7 mm diameter) was placed face down on the leaf, adjacent to the mid-rib approximately 1/3 of the distance from the petiole to the leaf apex. For the oxalate sample group, 2 mM oxalic acid was infiltrated via syringe at the same location on the leaf as the inoculum and mock inoculum sample groups.

Inoculated leaves were covered with a Petri dish lid and transferred to a Percival Model 818 incubator maintained at 22°C in the dark. Leaf pH was measured using a flat tip electrode (Cole-Palmer instrument Co., Vernon Hills, IL flat, sealed, epoxy body, BNC (EW-05990-65)) connected to a pH meter (Denver Instrument Co., Arvada, CO (Denver Instrument Basic pH meter), ~0.01 unit accuracy). Leaf pH was quantified at every two hours post inoculation

Detached leaf assay for metabolite analysis

In an independent experiment, detached leaves were inoculated as described for pH analysis for 'inoculated' and 'mock inoculated' sample groups (n=6 leaves per group). Tissue was collected at 0 (baseline), 16, 24, and 48 hours post inoculation using a hole punch (1 cm diameter). Initial experiments were performed to determine the dry weight of each punch, ~2.5 mg. A total of five leaf punches were taken adjacent to the necrotic lesion on the leaf to determine metabolic changes in healthy looking tissue (~12.5 mg dry weight). Leaf punches were immediately transferred to a 2.0 mL micro centrifuge tube containing stainless steel beads and flash frozen in liquid nitrogen (Fig.2). Tissue was stored at -80°C until further analysis.

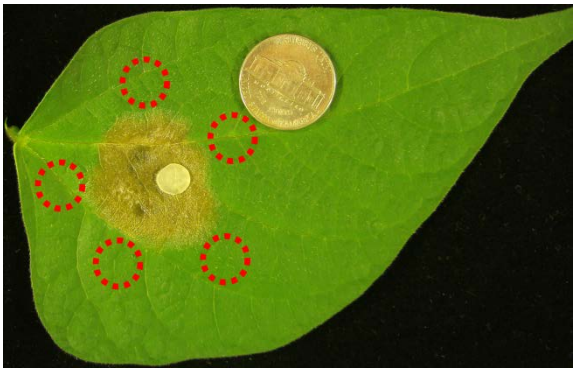

Figure 2: Detached leaf of bean line A195 at 48 hpi. The inoculum plug was placed adjacent to the central leaf axis. Dashed circles indicate approximate location and size of tissue taken for metabolite analysis. Nickel shown to for size.

Metabolite Extraction

Metabolites were extracted from collected leaf tissue to evaluate response of metabolic profiles in A195 and Sacramento to virulent and mock inoculation. Prior to metabolite extraction, previously frozen tissue was ground to a fine powder using six 2.3 mm diameter steel beads in a TissueLyser II produced by Qiagen. One mL of methanol:water (80:20, v:v) was

added to the ground leaf tissue, samples were shaken on a vortex mixer at high speed for two hrs at room temperature, and then centrifuged at 10,000 g for 10 min at 4° C. Extracts were transferred to 96 well plates for analysis using UPLC-MS, LC-MS/MS and GC-MS. The same metabolite extracts were used for targeted (UPLC-MS/MS) and non-targeted (UPLC-MS, GC-MS) metabolomic workflows. LC-MS/MS was performed on a Waters Xevo TQ-S triple quadrupole mass spectrometer coupled to a Waters M-class μAcquity UPLC system. LC-MS was performed on a Waters Acquity UPLC (C18 column (1.0 x 100 mm)) coupled to a Waters Xevo G2 QTOF. GC-MS used a Thermo Trace GC Ultra coupled to a Thermo ISQ mass spectrometer. All mass spectrometry instruments were used at the Proteomic and Metabolomics Facility, Colorado State University, Fort Collins CO (pmf.colostate.edu).

Targeted Metabolomics to detect phytohormones using UPLC-MS/MS

LC-MS/MS was used to evaluate the presence and absolute abundance of known defense metabolites. Multiple reaction monitoring (MRM) is a "targeted" detection procedure that allowed for the accurate quantitation of metabolites of extremely low abundance. MRM is a technique in mass spectrometry that utilizes metabolites that have been characterized for parent masses and fragmentation properties to enhance specificity and sensitivity in analysis using ion monitoring techniques [31] [32]. MRM assays focus directly a set of metabolites, excluding all other [32].

Targeted MRM selectively fragments metabolites of interest, and then quantified a single fragment of the spectrum. For MRM analysis, two leaf samples were pooled for a total of ten punches for metabolite extraction (~25 mg). Pooled leaf extracts were evaporated by

centrifugation under reduced pressure and resuspended in 100 µl of methanol spiked with internal standard. Internal standards are the deuterated version of the monitored phytohormone and are used to control for sample variation and possible ionization suppression. Spiked internal standards consisted of 50ng/ml of $d_5$jasmonate, $d_6$abscisic acid, $d_3$dihydrophaseic acid, $d_3$phaseic acid, and 200ng/ml of $d_2$salicylic acid. To conduct an MRM assay, a commercial standard of the compound of interest was run before experimental testing to assess parent (MS1) and product (MS2) ions.

Tandem mass spectrometry coupled to liquid chromatography (LC-MS/MS) was performed on a Waters Xevo TQ-S triple quadrupole mass spectrometer coupled to a Waters M-class µAcquity UPLC system by the Proteomic and Metabolomic Facility at Colorado State University. Multiple reaction monitoring transitions, including parent ion, cone voltage, collision energies, and fragment ions, were optimized by direct infusion of the phytohormones into the mass spectrometer. For LC-MS/MS analysis, a gradient was formed to separate phytohormones using buffer A, water with 0.1% formic acid, and buffer B, acetonitrile with 0.1% formic acid. The gradient was as follows: time (t) 0 minutes (m), 10% B; t 0.5 m, 10% B; t 8 m, 97% B; t 9 m, 97% B; t 9.5 m, 10% B; t 14 m, 10% B. The separation column was a reverse phase Waters three µM Atlantis dC18 (300 µM x 150 mm). Flow rate was 11.5 µL/min and column temperature was held at 40° C. Autosampler temperature was held at 4° C and injection volume was 1 µL. Phytohormones were analyzed in a single LC-MS run by utilizing polarity switching. Dwell times were 10 ms.

Capillary voltage was 3.2 kV in positive ion mode and -2.2 kV in negative ion mode. Desolvation temperature was 225° C, desolvation gas flow was 825 L/hr, cone gas flow was 150

L/hr, and nebulizer gas flow was 7.0 Bar. The collision gas used was argon and at 0.2 mL/min. Targeted metabolomics allowed for the accurate quantitation of selected metabolites of extremely low abundance.

Data was analyzed and phytohormones quantified for absolute abundance using Waters TargetLynx software. Phytohormones were quantified using the formula: analyte peak area x (internal standard concentration / internal standard peak area). The MRM assay allowed for absolute quantitation of metabolites due to the addition of internal standards and a standard calibration curve. Absolute quantitation for each compound was determined by the mean peak area of the MS2 (product ion) among replicate injections (n=3) and fit to a standard curve generated with commercially available internal standards. Quantification of leaf extract samples and quality controls were done using linear regression against a standard curve in TargetLynx (Waters).

Non-targeted metabolomics of leaf extracts using UPLC-TOF-MS and GC-MS.

A non-targeted metabolomics workflow was utilized in addition to targeted metabolomic analysis. Non-targeted metabolomics used both ultra-high performance liquid chromatography coupled to time of flight mass spectrometry (UPLC-TOF-MS), and gas chromatography mass spectrometry (GC-MS). Non-targeted UPLC-TOF-MS and GC-MS were used to detect a broader spectrum of metabolites compared to targeted metabolomics because they monitored all ions.

The UPLC-MS method used in this study can detect many amines/amino acids, flavonoids, phospholipids, sterols, terpenoids, organic acids, and secondary metabolites. Total leaf extracts were aliquoted into 200 µL samples for UPLC-MS. All aliquots were placed into a 96 well plate accordingly with six replicates per treatment. UPLC-MS methods were duplicated from previous methods described in, "Application of non-targeted metabolite profiling to discover novel markers of quality traits in an advanced population of malting barley" [33]. One µL of metabolite extract was injected on a Waters Acquity UPLC T3 column (1.8 µM, 1.0 9 100 mm) coupled to a Waters Xevo G2 Q-TOF-MS in duplicated injections. A gradient from solvent A (water, 0.1% formic acid) to solvent B (acetonitrile, 0.1% formic acid) was used to achieve separation. Injections were made in 100% A, 100% A held for 1 min and followed a linear gradient to 95% B in 12 min and re-equilibrated for 3.95 min (total run time of 30 min/sample). Flow rate was maintained at 200 µL/min for the duration of the run, the column was held at 50 °C, and samples were held at 5 °C. Column eluate was infused into a Waters Q-TOF-MS fitted with an electrospray source. Data was collected in positive ion mode, scanning from 50–1200 m/z at a rate of 1 scan per second with 0.1 s interscan delay. Calibration was performed prior to sample analysis via infusion of sodium formate solution, with mass accuracy within 5 ppm. The capillary voltage was held at 2200 V, the sample cone at 30 V, the source temp at 130 °C, and the desolvation temperature at 300 °C with a nitrogen desolvation gas flow rate of 400 l/hr. [33].

The GC-MS method used in this study can detect ureides, alkaloids, amide sugars, amine/amino acids, fatty acids, organic acids, sterols, and mono- di- and trisaccharides. Total leaf extracts were aliquoted into 350 µL samples for GC-MS. All aliquots were placed into a 96

well plate accordingly. Each treatment had six replicates. GC-MS samples were evaporated by centrifugation under reduced pressure until absolutely no water was present in the well, as water inhibits the derivatization reactions required to volatilize primary metabolites. Samples were derivatized (methoximation and silylation) by adding 50 µL of pyridine containing 15 mg/mL of methoxyamine briefly vortexting, and incubated at 60°C for 45 minutes, sonicated for 10 minutes in a water bath and incubated again at 60°C for 45 minutes. Next, 50 µL of N-methyl-N-trimethylsilytrifluoroacetamide w/1% trimethylchlorosilane (MSTFA + 1% TMCS) solution was then added to the samples, the sample were briefly vortexed and incubated for another 30 minutes at 60°C. One µL of derivatized samples were injected in duplicate into the GC-MS as previously described [19], [34], [35] using a Thermo Trace GC Ultra coupled to a Thermo ISQ mass spectrometer (Thermo Scientific). Briefly, 1 µL of each sample was injected in a 1:10 split ratio twice in discrete randomized blocks (n=2 injections per sample). Separation occurred using a 30 m TG-5MS column (Thermo Scientific, 0.25 mm i.d., 0.25 µm film thickness) with a 1.2 mL/min helium gas flow rate, and the program consisted of 80 °C for 30 sec, a ramp of 15 °C per min to 330 °C, and an 8 min hold. Masses between 50-650 m/z were scanned at 5 scans/sec after electron impact ionization.

Raw files generated from the mass spectrometers (.raw for GC-MS and .D for LC-MS) were converted to .cdf format for processing in XCMS [36]. XCMS was used to create a matrix of molecular features as defined by retention time and mass (m/z) for UPLC-MS and GC-MS non-targeted metabolomics profiling. Spectra was normalized to total ion current (TIC), and the relative quantity of each molecular feature determined by the mean area of the

chromatographic peak among two replicate injections. UPLC-MS data further grouped features into spectral clusters using the R package RAMClust [37].

Identification of metabolites was performed by matching molecular features or clusters against in-house and external metabolite databases including Metlin [38], MassBank [39] and lab developed libraries using NIST (National Institute of Standards Technology) MS search program.

Statistical Analysis

Statistical tests included both univariate and multivariate statistical models using R statistical software v3.1.2 [40] [41] [42] [43], JMP (v11.0, SAS Institute, Cary, NC) [44], and SIMCA P+ (v12.0, Umetrics, Sweden) [45]. Transpiration rate, photosynthetic rate, and stomatal conductance data were analyzed using analysis of variance (ANOVA)(X~ line x treatment) and contrasts in R Statistical Software. Leaf pH was compared using Student's t test with a p-value threshold of 0.05 for each line, at each time point compared to the mock inoculation control. Leaf pH was also compared via contour plots generated in JMP software.

Metabolite analysis utilized both univariate and multivariate statistical models to characterize metabolites associated with resistant and susceptible lines. Samples that injected poorly into the mass-spectrometry instruments were considered outliers and removed from the analysis. The following samples were excluded from analysis; two replicates from A195 time point 0, one virulent inoculated replicate from A195 and Sacramento at 16hpi, two mock replicates from A195 24hpi, and one virulent inoculated replicate from Sacramento 48hpi.

Univariate statistics included Student's t-test ($p \leq 0.05$), and z-transformation, to compare treatment and control means. Significance for z-scores was based on the empirical rule, meaning greater than or equal to 1.96 and less than or equal to -1.96 z-scores were considered significant [46]. Orthogonal projection to latent structures- discriminant analysis (OPLS-DA)), a multivariate statistical model, was used to determine segregating metabolites by setting classes A195 mock, A195 inoculated, Sacramento mock, and Sacramento inoculated at each time point. Data analysis utilized multiple statistical software programs and models to evaluate bean response to virulent and mock inoculum.

Pathway analysis with Pathway Studio

Major contributing pathways associated with identified metabolites were determined using Pathway Studio Plant Web (software to help better understand biological relationships and mechanisms, Elsevier, Amsterdam, Netherlands) in conjunction with SoyCyc (soybean metabolic database) [47]. Gene Set Enrichment Analysis (GSEA) was conducted in Pathway Studio Plant Web using the Mann-Whitney-U Test, and significantly enriched pathways were determined using a threshold of $p < 0.05$ [48]. SoyCyc and Pathway Studio were utilized to assess pathways contributing to resistance or susceptibility in A195 and Sacramento to white mold.

CHAPTER THREE / RESULTS AND DISCUSSION

A195 contains greater quantitative resistance to Sclerotinia compared to Sacramento

Resistance to Sclerotinia for A195 and Sacramento was determined using the Petzoldt and Dickson Straw Test [29]. The score for A195 was 4.9 ± 0.2, and was significantly lower than Sacramento at 6.4 ± 0.3 (Student's t-test, $p < 0.05$). In the release of A195, Singh reported a mean Straw Test scores of 3.7 in greenhouse trials and 2.0 in field trials [23]. While the scores observed in this study were higher than other reports, the data support A195 contains greater resistance to Sclerotinia than Sacramento, a susceptible cultivar.

Physiological resistance to Sclerotinia encompasses reduced variation in photosynthetic traits and leaf surface pH

Oxalic acid (OA), is a dicarboxylic acid and is the main secreted pathogenicity factor for Sclerontia ssp., travels ahead of the infecting mycelia and supports pathogen growth by (i) reducing leaf pH and (ii) inducing stomata to remain open [13]. In addition to stimulating enzyme synthesis within Sclerotinia, OA results in programed cell death in plant tissue to induce necrosis prior to colonization of the fungus. [4]. Oxalic acid reduces the leaf pH which allows for acidity-induced activation of pectolytic enzymes such as polygalacturonase, a plant cell wall degrading enzyme [4, 12] [7]. Polygalacturonase and OA together elicit phytoalexin biosynthesis, a plant defense [49]. In addition, OA induces stomatal opening by inducing the breakdown of starch in guard cells and allowing the accumulation of K^+ [13, 15]. Open stomata

allow for secondary infection from mycelia and increase pathogen growth rate on plant tissues. [4, 12]. Guimarães and Stoz [9] found that when broad bean was infected with *S. sclerotiorum,* stomatal opening preceded fungal colonization, allowing hyphae to enter the leaf through stomata causing secondary colonization. In fact, stomatal entry was found to be more common than penetration through the cuticle by hyphae. Further, hyphae do not ingress until the leaf tissue has reached a reduced pH for infection, approximately 4.0. [4]. Thus, infection by Sclerotinia is associated with a rapid drop in pH and increased stomatal conductance and transpiration rates [13].

Gas exchange characteristics were measured using the LI-6400 photosynthesis system at 48 hours post inoculation with either the mock or virulent inoculum. Transpiration rate, stomatal conductance, and photosynthetic rate decreased in A195 post inoculation with virulent inoculum compared to mock inoculum (Student's t-test, $p < 0.05$, Fig 3). There was no change in Sacramento for either mock or virulent inoculum (Fig. 3). These results indicate that Sacramento and A195 differ in transpiration rate, stomatal conductance, and photosynthetic rate when challenged with virulent, but not mock inoculum.

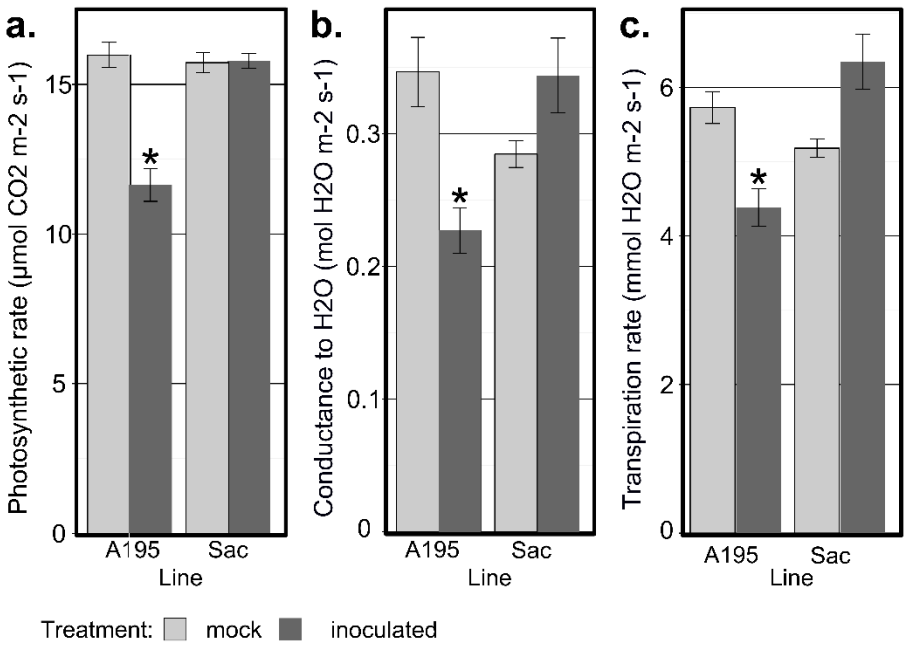

Figure 3: Photosynthetic rate, stomatal conductance, and transpiration rate for A195 and Sacramento leaves after mock and virulent inoculum. Mock treatment in light grey and inoculated treatment in dark grey with genotype along the x axis. * Significant at p = 0.05.

Stomatal closure is a potential mechanism of resistance to secondary colonization in A195. The gas exchange experiment revealed that the resistant line A195 had reduced stomatal conductance, photosynthetic rate, and transpiration rate post inoculation, whereas Sacramento exhibited no change in any of the physiological traits. The decreased levels of these traits may indicate that stomata close in response to Sclerotinia infection in A195, but not in Sacramento. To validate this concept, future work can include microscopy to visualize stomatal closure in resistance and susceptible lines.

Leaf pH experiments utilized a detached leaf with virulent, avirulent, or mock inoculum. Direct oxalic acid infiltration was also utilized to test the effect of oxalic acid alone on leaf pH. Optimal leaf pH for the activation of pathogenic enzymes to break down plant tissue is pH = 4.0. Leaf pH did not decrease to optimal pH when leaves were inoculated with mock inoculum for

either A195 or Sacramento (data not shown). There was a decrease in leaf pH over time in both A195 and Sacramento in response to virulent inoculum (Fig 4). Not only did leaf pH drop over time, but a pH gradient radiated out from the site of virulent inoculation in the leaf (Fig. 4). Leaf pH dropped to 4 in Sacramento by 24 hpi, however, in A195 leaf pH did not reach 4 until 48 hpi. The slower response in leaf pH change to optimal values for enzyme activation in the pathogen supports that a portion of the resistant response in A195 is the ability to slow the reduction in leaf pH following infection.

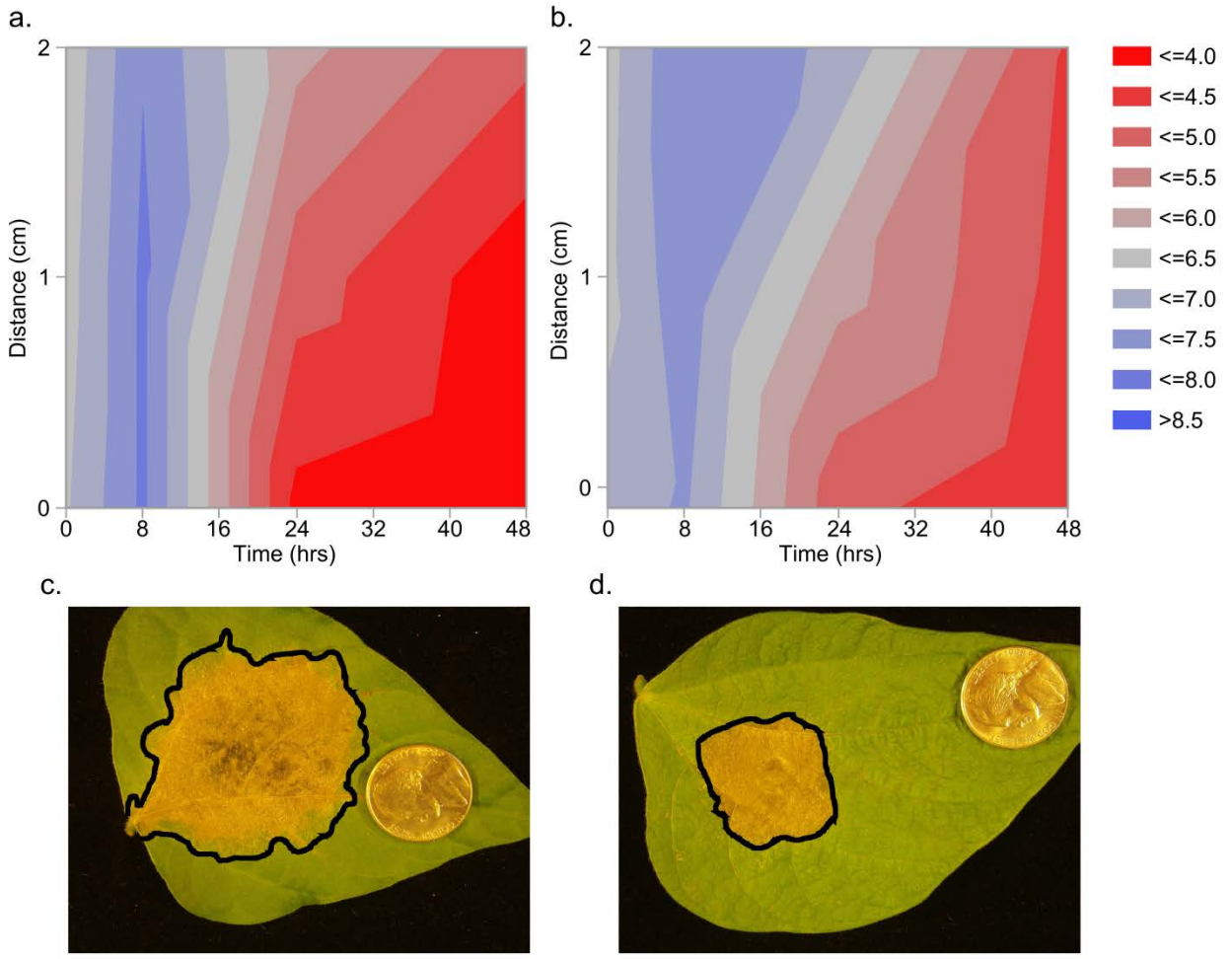

Figure 4: Change in leaf tissue pH 24hpi at 0, 1, and 2 cm from the infection site by virulent inoculum for Sacramento (a) and A195 (b) bean lines. Contour plots were used to plot pH in response to time and distance and were generated using n=3 replicates per sample group. Necrotic lesion at 24hpi, outlined in black, from the virulent inoculum site on Sacramento (c) and A195 (d) leaves. Nickel for size reference.

Leaves were also inoculated with an avirulent oxalic acid deficient mutant to evaluate leaf pH changes. Leaf pH did not decrease in either A195 or Sacramento when leaves were inoculated with avirulent inoculum (Fig. 5). There was no effect of the avirulent inoculum on leaf pH changes in either bean line. These results indicate that oxalic acid is necessary for the reduction of leaf pH.

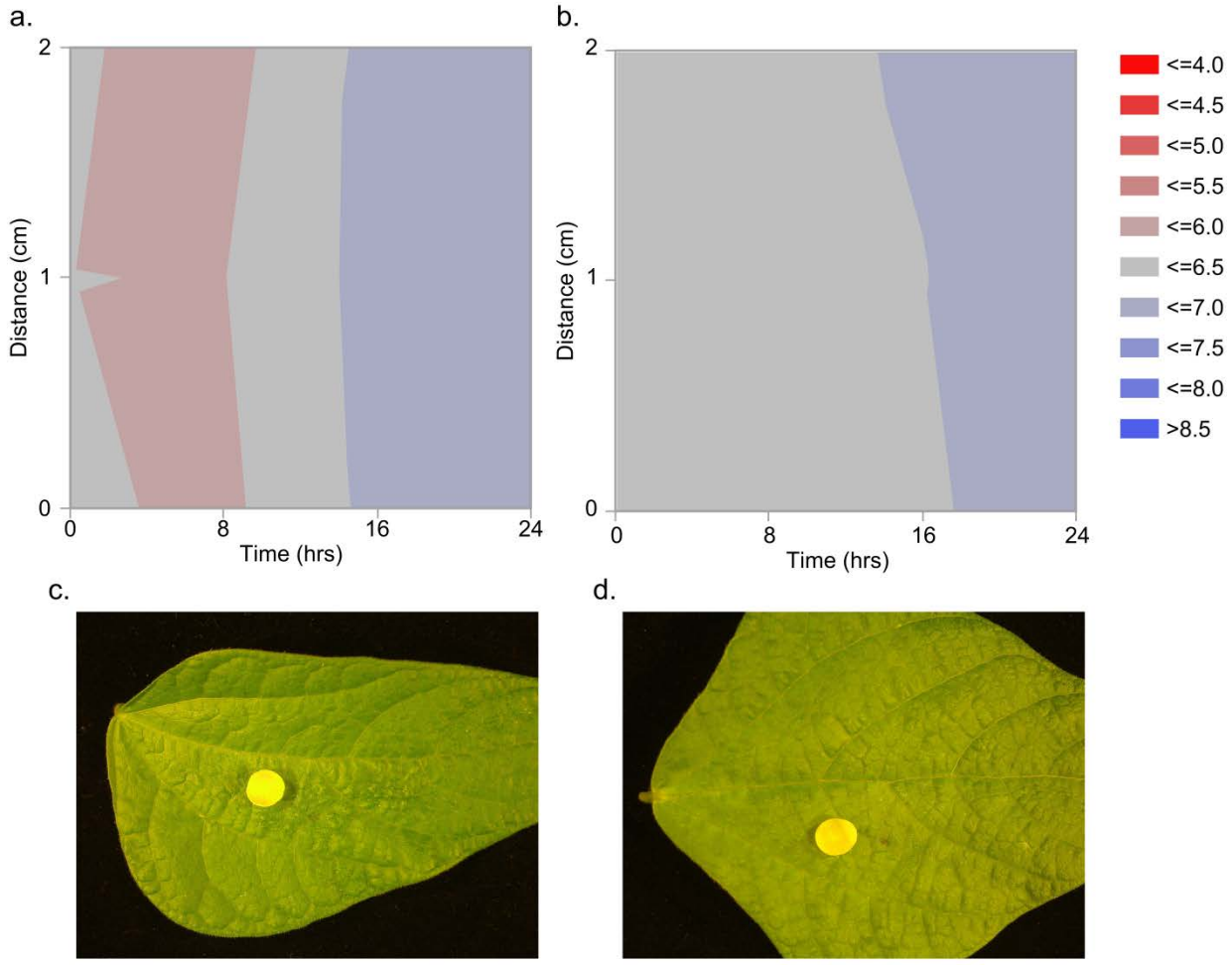

Figure 5: Change in leaf tissue pH 24 hpi at 0, 1, and 2 cm from the infection site by avirulent inoculum for Sacramento (a) and A195 (b) bean lines. Contour plots were used to plot pH in response to time and distance and were generated using n=3 replicates per sample group. Necrotic growth 24hpi from the avirulent inoculum site on Sacramento (c) and A195 (d) leaves.

Leaves were also infiltrated with 2mM oxalic acid to evaluate if A195 and Sacramento responded similarly to the presence of oxalic acid alone. Leaf pH dropped rapidly in both A195 and Sacramento. Sacramento decreased to pH= 4 within the first 30 min after oxalic acid infiltration. However, the leaf pH in A195 dropped to pH 4 at approximately 1 hpi and at 4 cm from the inoculation site, it never dropped to pH = 4 (Fig. 6). These results clearly show a slower response to reduced pH in A195 compared to Sacramento due to oxalic acid exposure indicating that oxalic acid is sufficient for the reduction of leaf pH but that there is variation in the rate of pH reduction between A195 and Sacramento.

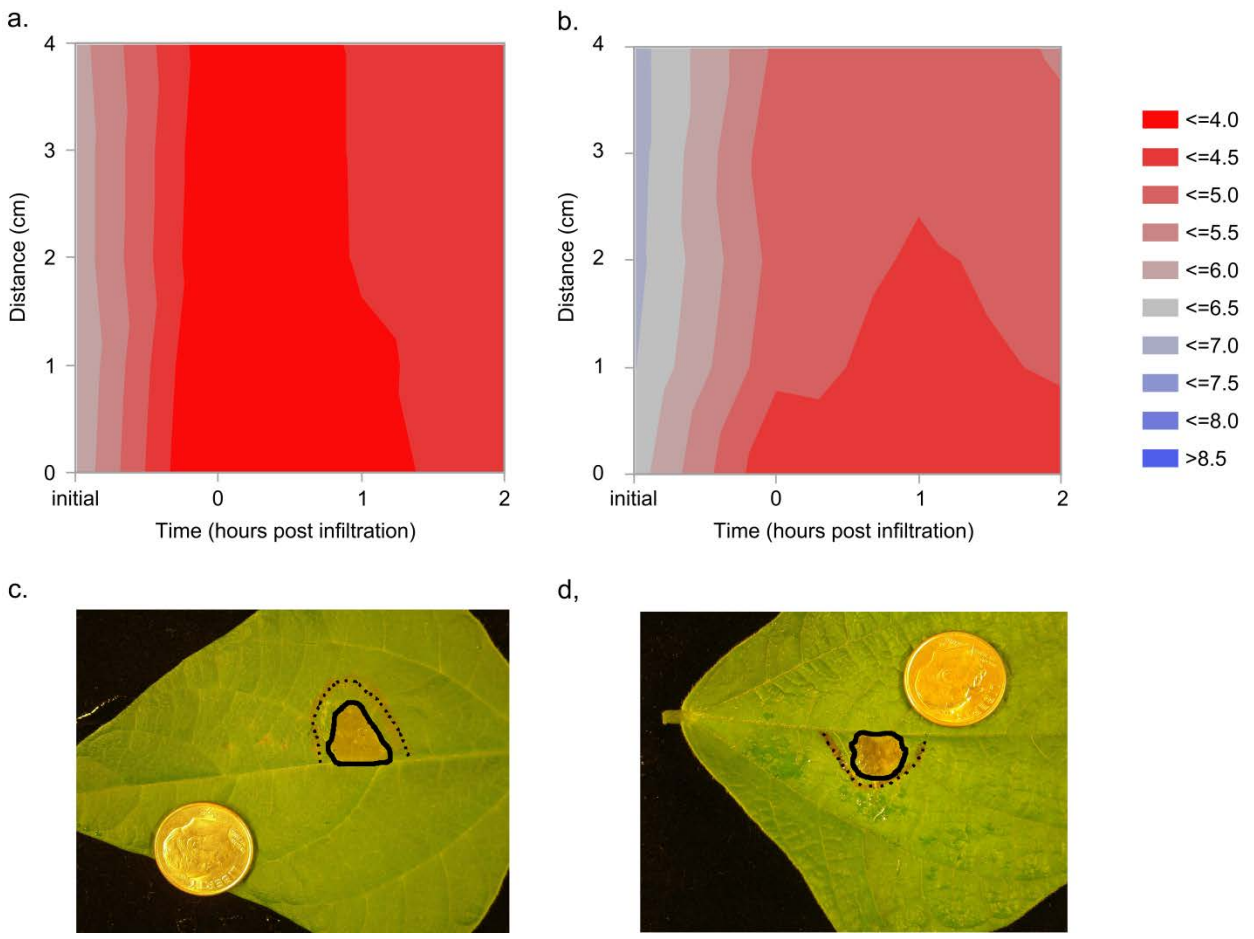

Figure 6: Change in leaf tissue pH 24 hpi at 0, 1, and 2 cm from the infiltration with 2 mM oxalic acid for Sacramento (a) and A195 (b) bean lines. Contour plots were used to plot pH in response to time and distance and were generated using n=3 replicates per sample group. Necrotic growth 24hpi from the avirulent inoculum site on Sacramento (c) and A195 (d) leaves. Dotted line represents area of infiltration. Nickle for size reference.

The pH levels observed in this study demonstrated that oxalic acid is both necessary and sufficient to lower the pH in leaves in response to virulent inoculum. Leaves inoculated with virulent inoculum displayed a decrease in pH over the course of infection in both A195 and Sacramento, however, the decrease was faster in Sacramento compared to A195. Leaf pH dropped over the time course of infection and a pH gradient radiated out from the virulent inoculation site. No change in leaf pH occurred when inoculated with avirulent inoculum

(oxalate deficient mutant) supporting that oxalic acid is necessary for the reduction in leaf pH during pathogenesis. In this experiment, a direct oxalic acid infusion resulted in an acidic environment in the absence of *S. sclerotiorum* demonstrating that oxalic acid is a causal agent for the drop in leaf pH.

The bean line A195 reduced the rate of change in pH to an optimal level (pH =4) for necrosis to occur in response to *Sclerotinia sclerotiorum*. The reduced change in A195 may have been accomplished by the breakdown of chitin to n-acetylglucosamine monomers, which buffers the reduction in pH [50]. Endochitinases are an inducible defense response of plants to attack and partially digest fungal cell walls and produce monomers of n-acetylglucosamine [51] [52]. N-acetylglucosamine, an amide sugar, increased in A195 at 16 hpi compared to Sacramento in response to virulent inoculum. Since n-acetylglucosamine is more abundant in A195 compared to Sacramento, it possible that n-acetylglucosamine may buffer the acidic pH produced by OA in resistant genotypes. N-acetylglucosamine, a fungal wall fragment, is also an active inducer of plant defense responses [53].

Phytohormones exhibited minor variation between A195 and Sacramento

Phytohormones are essential for systemic and induced defense signaling to protect plants against invading pathogens [5]. Guo et al. found that the phytohormones jasmonic acid, salicylic acid, and ethylene used a signaling response as a plant defense against *S. sclerotiorum.* Phytohormones were detected in this study using multiple reaction monitoring via a Waters Xevo TQ-S triple quadrupole mass spectrometer coupled to a Waters M-class µAcquity UPLC

system with deuterated internal standards. The phytohormones targeted in this study included abscisic acid, dihydrophaseic acid, phaseic acid, gibberellic acid, jasmonic acid, methyl-jasmonic acid, salicylic acid, methyl-salicylic acid, indole-3-buteric acid and tranzeatin.

Some phytohormones varied differently in A195 compared to Sacramento between virulent and mock inoculum treatments. Only dihydrophaseic acid, phaseic acid, and abscisic acid were detected (Table 1). Abscisic acid and dihydrophaseic acid did not change in either A195 or Sacramento in response to virulent inoculation. Phaseic acid abundance increased in Sacramento by ~188.8 ng/ml in response to virulent inoculum, however, it did not change in A195. Phaseic acid and dihydrophaseic acid are inactivated forms of the hormone abscisic acid (Fig. 7), however, neither hormone show an affinity for stomatal closure in *Vicia faba* (L.) [54]. Abscisic acid and dihydrophaseic acid did not change in either A195 or Sacramento in response to virulent inoculation. Phaseic acid increased in Sacramento in response to virulent inoculation, however, it did not change in A195.

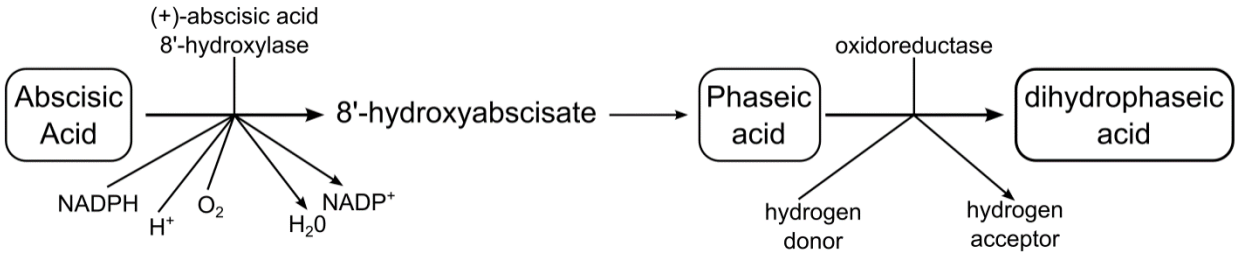

Figure 7: Abscisic acid derivative biosynthesis resulting in phaseic acid and dihydrophaseic acid [47].

Several phytohormone precursors were also detected in the non-targeted metabolomics experiment (results discussed below). Specifically, the fatty acid α-linolenic acid increased in A195 at 48 hpi when exposed to virulent inoculum compared to mock inoculum, however, it did not change in Sacramento. This fatty acid is a precursor to jasmonic acid [55], a phytohormone that is known to enhance resistance to necrotrophic pathogens [5]. Jasmonates are also a vital plant phytohormone that regulate defense responses [56]. These studies suggest that α-linolenic acid is associated with resistance in common bean, therefore could be associated with disease resistance.

Table 1: Phytohormone absolute quantitation of abscisic acid, phaseic acid, and dihydrophaseic acid in detached leaves of A195 inoculated with mock and virulent inoculum.

		A195 vs Sacramento		A195				Sacramento			
Phytohormone	**hpi**	**mock p-value†**	**inoc p-value†**	**inoc vs. mock p-value†**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score††**	**inoc vs. mock p-value†**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score††**
gibberellin a37 glucosyl ester, putative LC-MS	0	0.63									
	16	0.01	0.70	0.05	14.58±1.2	39.52±9.81	8.53	0.65	23.96±2.87	29.93±13.6	0.85
	24	**<0.01**	**0.02**	0.07	52.44±21.1	19.78±4.34	-0.77	0.25	28.74±4.99	66.78±30.66	3.11
	48	0.42	0.38	0.73	34.16±5.78	30.58±5.59	-0.25	0.51	28.75±7.09	55.85±39.64	1.56
methyl 7-epi-12-hydroxyjasmonate glucoside, putative LC-MS	0	0.01									
	16	0.02	0.09	0.46	263.04±13.17	146.94±62.87	-3.60	**0.02**	**174.41±28.41**	**77.49±13.53**	**-1.39**
	24	**<0.01**	**<0.01**	0.27	163.67±25.48	252.76±38.03	1.75	0.07	102.63±7.58	153.88±24.12	2.76
	48	0.00	0.07	0.16	225.55±36.18	157.58±32.79	-0.77	0.51	186.01±72.06	128.63±24.52	-0.33
abscisic acid	16	0.22	0.22	0.81	73.33±41.41	61.94±19.36	-1.79	0.06	16.78±5.03	34.50±5.92	7.05
phaseic acid	16	**0.01**	0.16	0.70	763.28±168.84	650.63±228.47	1.54	**0.02**	**94.48±36.64**	**283.28±47.95**	**-7.87**
dihydrophaseic acid	16	0.83	0.18	0.17	6588.18±1100.75	15504.63±5605.92	-3.18	0.79	7895.33±2679.63	6118.80±2679.63	-0.15

† p-value

‡ Arbitrary Unit (ABU)

Metabolomic profiles differed between A195 and Sacramento in response to *S. sclerotiorum* infection

A non-targeted approach was used to evaluate metabolic profiles of A195 and Sacramento in response to virulent and mock inoculum. Non-targeted metabolomics results in relative abundance for all detectable metabolites. The non-targeted metabolic approach used GC-MS to detect 10,285 features (mass/charge (z) _retention time) and LC-MS to detect 4,292 clusters (group of features predicted to a single metabolite). UPLC-MS detected amines/amino acids, flavonoids, lipids, organic acids, and secondary metabolites. GC-MS detected ureides, alkaloids, amide sugars, amine/amino acids, fatty acids, organic acids, sterols, and sugars. A multivariate supervised analysis, orthogonal projection to latent structures-discriminant analysis (OPLS-DA) was used to characterize variation in metabolite profiles among sample types. The OPLS-DA resulted in clear separation between lines in the overall metabolome at time point zero explaining 99% of the variation. There was also clear difference in the metabolome of A195 and Sacramento when challenged with virulent inoculum with component 2 explaining 58% of the variation at 16 hpi, component 1 explaining 44% of the variation and component 2 84% of the variation at 24 hpi, and component 1 explaining 45% of the variation and component 2 explaining 61% of the variation at 48 hpi (Fig. 8). Differing metabolomes in response to virulent inoculum suggests a portion of metabolites may be part of a resistance or susceptibility mechanism in A195 and Sacramento, respectively.

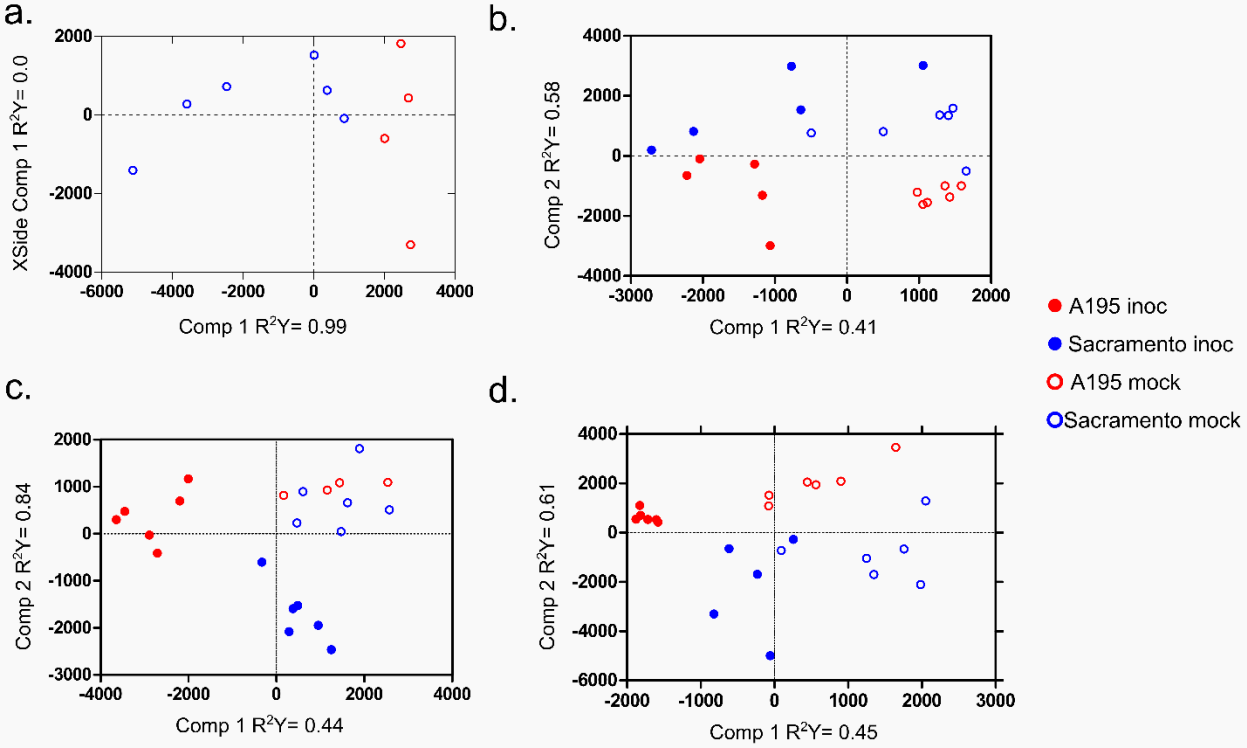

Figure 8: Discrimination of leaf metabolome using OPLS-DA at time points zero (basal leaf tissue) (a), 16 hpi (b), 24 hpi (c), and 48 hpi (d) for lines, and inoculum treatments.

Among the detected features (GC-MS) and clusters (LC-MS), 140 metabolites varied in at least 1 time point in relative abundance between A195 and Sacramento relative to their mock controls via (studentized t-test, $p < 0.05$). The features and clusters included important primary metabolite classes such as amines, organic acids, and sugars (Appendix Table 1). Some metabolites were detected on both GC-MS and LC-MS platforms such as leucine, phenylalanine, proline, and ferulic acid. Metabolites found on both platforms showed similar trends with the exception of ferulic acid. While the abundance of ferulic acid differed between A195 and Sacramento based on analysis with LC-MS, not change was observed for this compound by GC-MS. Many secondary metabolites such as flavonoids and phytoalexins differed in abundance between A195 and Sacramento in response to virulent inoculum. Overall, differing changes were observed in large metabolomic classes between A195 and Sacramento in response to virulent inoculum and may be related to resistance or susceptibility in the two lines.

Metabolic profiles also differed between A195 and Sacramento in response to virulent versus mock inoculum. The majority of changing metabolites responded at 16 hpi with virulent inoculum in A195 compared to the mock inoculum. At 24 hpi and 48 hpi, metabolite abundance differences between Sacramento and A195 were much lower (Fig 9). Metabolic profiles respond differently in A195 and Sacramento in response to *S. sclerotiorum*. A195 expressed more responding metabolites at 16 hpi with virulent inoculum compared to Sacramento. Responding metabolites included primary metabolites like amino acids and sugars and secondary metabolites, such as, phytoalexins and flavonoids. More changing metabolites at 16 hpi in A196 suggests that a portion of the resistance response in A195 is the ability to quickly change metabolites in response to virulent inoculum.

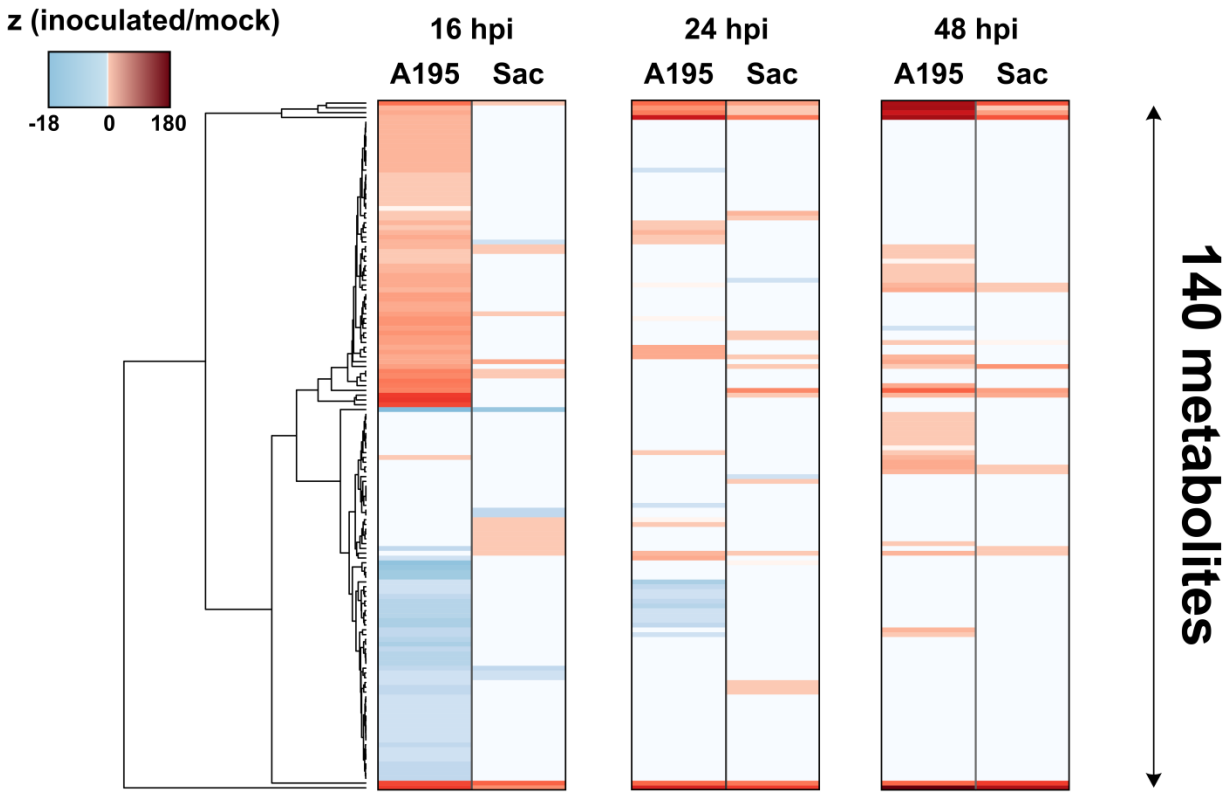

Figure 9: A heat map of z scores highlights groups of metabolites that either increased or decreased in A195 and Sacramento over a time course of 16, 24, and 48 hpi with virulent inoculum.

Pathway Studio

Major contributing pathways were determined using Pathway Studio Plant Web (software to help better understand biological relationships and mechanisms, Elsevier) which utilizes AraCyc (*Arabidopsis thaliana*), RiceCyc (rice), and MaizeCyc (maize) metabolic databases [47]. Pathway Studio was operated using the 140 varying metabolites found between A195 and Sacramento in an enrichment analysis including AraCyc, RiceCyc and MaizeCyc. Out of the 140 metabolites, 49 metabolites were successfully mapped to 48 pathways.

The top-5 ranked enriched *Arabidopsis* signaling pathways generated by GSEA in Pathway Studio with mapped metabolites differing between A195 and Sacramento were as follows; seed development ABA, guard cell ABA, ethylene, salicylic acid, and jasmonic acid signaling (Table 2).

Table 2: Top-5 ranked enriched *Arabidopsis* signaling pathways generated by GSEA in Pathway Studio with mapped metabolites differing between A195 and Sacramento.

Name	# of Entities	Expanded # of Entities	Overlap	Percent Overlap	Overlapping Entities	p-value†Error! ookmark not defined.	Jaccard similarity§
Seed Development ABA Signaling	58	58	2	3	D-glucose, sucrose	2.79E-2	1.90E-2
Guard Cell ABA Signaling	71	141	2	1	malate, sucrose	1.43E-1	1.06E-2
Ethylene Signaling	45	45	1	2	D-glucose	2.06E-1	1.08E-2
Salicylic Acid Signaling	70	73	1	1	Lipids	3.17E-1	8.26E-3
Jasmonic Acid Signaling	63	98	1	1	Lipids	4.06E-1	6.85E-3

§ Ratio of the number of matching elements to the total pairs of elements ignoring zeros.

The top-10 ranked enriched AraCyc pathways generated by GSEA in Pathway Studio with mapped metabolites differing between A195 and Sacramento were as follows; tRNA charging pathway, Indole-3-acetyl-amino acid biosynthesis, superpathway of IAA conjugate biosynthesis, superpathway of threonine biosynthesis, homoserine biosynthesis, lysine biosynthesis, superpathway of isoleucine biosynthesis, jasmonoyl-amino acid conjugates biosynthesis I, asparagine biosynthesis I, superpathway of phenylalanine, and tyrosine and tryptophan biosynthesis (Table 3)

Table 3: The top-10 ranked enriched AraCyc pathways generated by GSEA in Pathway Studio with mapped metabolites differing between A195 and Sacramento.

Name	# of Entities	Expanded # of Entities	Overlap	Percent Overlap	Overlapping Entities	p-value†	Jaccard similarity§
tRNA charging pathway	83	126	14	11	L-glutamate, L-glutamine, L-serine, L-asparagine, L-tyrosine, L-leucine, L-glycine, L-isoleucine, L-alanine, L-threonine, L-phenylalanine, L-aspartate, L-valine, L-tryptophan	6.04E-15	8.70E-2
Indole-3-acetyl-amino acid biosynthesis	25	25	7	28	L-glutamate, L-glutamine, L-alanine, L-leucine, L-phenylalanine, L-aspartate, L-valine	7.53E-11	1.04E-1
Superpathway of IAA conjugate biosynthesis	31	41	7	17	L-glutamate, L-glutamine, L-alanine, L-leucine, L-phenylalanine, L-aspartate, L-valine	3.28E-9	8.43E-2
Superpathway of threonine biosynthesis	24	46	7	15	L-serine, L-isoleucine, L-alanine, L-threonine, L-leucine, L-aspartate, L-valine	7.64E-9	7.95E-2
Homoserine biosynthesis	19	36	6	16	L-serine, L-isoleucine, L-alanine, L-leucine, L-aspartate, L-valine	5.62E-8	7.59E-2
Lysine biosynthesis	32	61	7	11	L-glutamate, L-serine, L-isoleucine, L-alanine, L-leucine, L-aspartate, L-valine	5.83E-8	6.79E-2
Superpathway of isoleucine biosynthesis	45	121	8	6	L-glutamate, L-serine, L-leucine, L-isoleucine, L-alanine, L-threonine, L-aspartate, L-valine	4.66E-7	4.93E-2
Jasmonoyl-amino acid conjugates biosynthesis I	16	16	4	25	L-isoleucine, L-leucine, L-phenylalanine, L-valine	1.97E-6	6.55E-2
Asparagine biosynthesis I	9	19	4	21	L-glutamate, L-glutamine, L-asparagine, L-aspartate	4.15E-6	6.25E-2
Superpathway of phenylalanine, tyrosine and tryptophan biosynthesis	51	117	7	5	L-glutamate, L-glutamine, L-serine, L-tyrosine, L-phenylalanine, L-tryptophan, shikimic acid	5.18E-6	4.40E-2

The top-10 ranked enriched RiceCyc pathways generated by GSEA in Pathway Studio with mapped metabolites differing between A195 and Sacramento were as follows; tRNA charging pathway, IAA conjugate biosynthesis II, asparagine biosynthesis III, superpathway of citrulline metabolism, NAD salvage pathway I, asparagine biosynthesis I, citrulline biosynthesis, tryptophan biosynthesis, arginine degradation I (arginase pathway), and superpathway of leucine, valine, and isoleucine biosynthesis (Table 4).

Table 4: The top-10 ranked enriched RiceCyc pathways generated by GSEA in Pathway Studio with mapped metabolites differing between A195 and Sacramento.

Name	# of Entities	Expanded # of Entities	Overlap	Percent Overlap	Overlapping Entities	p-value†	Jaccard similarity§
tRNA charging pathway	83	126	14	11	L-glutamate, L-glutamine, L-serine, L-asparagine, L-tyrosine, L-leucine, L-glycine, L-isoleucine, L-alanine, L-threonine, L-phenylalanine, L-aspartate, L-valine, L-tryptophan	1.37E-15	8.69E-2
IAA conjugate biosynthesis II	18	28	5	17	L-glutamate, L-glutamine, L-alanine, L-leucine, L-aspartate	3.37E-7	6.94E-2
Asparagine biosynthesis III	11	21	4	19	L-glutamate, L-glutamine, L-asparagine, L-aspartate	4.29E-6	6.06E-2
Superpathway of citrulline metabolism	41	58	5	8	L-glutamate, L-glutamine, L-ornithine, L-aspartate, urea	1.39E-5	4.90E-2
NAD salvage pathway I	24	34	4	11	nicotinate, Nicotinamide, L-glutamate, L-glutamine	3.15E-5	5.06E-2
Asparagine biosynthesis I	12	40	4	10	L-glutamate, L-glutamine, L-asparagine, L-aspartate	6.08E-5	4.70E-2
Citrulline biosynthesis	29	41	4	9	L-glutamate, L-glutamine, L-ornithine, urea	6.71E-5	4.65E-2
Tryptophan biosynthesis	22	45	4	8	L-glutamate, L-serine, L-glutamine, L-tryptophan	9.72E-5	4.44E-2
Arginine degradation I (arginase pathway)	13	17	3	17	L-glutamate, L-ornithine, urea	9.79E-5	4.76E-2
Superpathway of leucine, valine, and isoleucine biosynthesis	36	87	5	5	L-glutamate, L-leucine, L-isoleucine, L-threonine, L-valine	1.00E-4	3.81E-2

The top-10 ranked enriched MaizeCyc pathways generated by GSEA in Pathway Studio with mapped metabolites differing between A195 and Sacramento resulted in; tRNA charging pathway, threonine biosynthesis, asparagine biosynthesis I, superpathway of asparagine biosynthesis, tyrosine biosynthesis II, superpathway of citrulline metabolism, phenylalanine biosynthesis II, citrulline biosynthesis, inosine-5'-phosphate biosynthesis II, and tryptophan biosynthesis (Table 5).

Table 5: The top-10 ranked enriched MaizeCyc pathways generated by GSEA in Pathway Studio with mapped metabolites differing between A195 and Sacramento.

Name	# of Entities	Expanded # of Entities	Overlap	Percent Overlap	Overlapping Entities	p-value†	Jaccard similarity§
tRNA charging pathway	84	127	14	11	L-glutamate, L-glutamine, L-serine, L-asparagine, L-tyrosine, L-leucine, L-glycine, L-isoleucine, L-alanine, L-threonine, L-phenylalanine, L-aspartate, L-valine, L-tryptophan	4.53E-15	8.64E-2
Threonine biosynthesis	30	70	8	11	L-glutamate, L-serine, L-leucine, L-isoleucine, L-alanine, L-threonine, L-aspartate, L-valine	4.86E-9	7.21E-2
Asparagine biosynthesis I	10	20	4	20	L-glutamate, L-glutamine, L-asparagine, L-aspartate	4.61E-6	6.15E-2
Superpathway of asparagine biosynthesis	12	22	4	18	L-glutamate, L-glutamine, L-asparagine, L-aspartate	6.89E-6	5.97E-2
Tyrosine biosynthesis II	14	23	4	17	L-glutamate, L-tyrosine, L-phenylalanine, L-tryptophan	8.31E-6	5.88E-2
Superpathway of citrulline metabolism	41	58	5	8	L-glutamate, L-glutamine, L-ornithine, L-aspartate, urea	1.96E-5	4.90E-2
Phenylalanine biosynthesis II	14	31	4	12	L-glutamate, L-tyrosine, L-phenylalanine, L-tryptophan	2.85E-5	5.26E-2
Citrulline biosynthesis	29	41	4	9	L-glutamate, L-glutamine, L-ornithine, urea	8.81E-5	4.65E-2
Inosine-5'-phosphate biosynthesis II	35	44	4	9	L-glutamate, L-glutamine, L-glycine, L-aspartate	1.17E-4	4.49E-2
Tryptophan biosynthesis	23	46	4	8	L-glutamate, L-serine, L-glutamine, L-tryptophan	1.39E-4	4.40E-2

Some of the top-10 ranked enriched pathways generated by GSEA in Pathway Studio with mapped metabolites differing between A195 and Sacramento for AraCyc, RiceCyc, and MaizeCyc overlapped. Two of the enriched metabolic pathways identified by Pathway Studio overlapped between AraCyc, RiceCyc, and MaizeCyc in response to virulent inoculum compared to mock. RiceCyc and MaizeCyc overlapped in three metabolic pathways in response to virulent inoculum compared to mock. AraCyc identified eight unique enriched metabolic pathways in response to virulent inoculum compared to mock. RiceCyc identified five unique enriched metabolic pathways in response to virulent inoculum compared to mock. MaizeCyc also identified five unique enriched metabolic pathways in response to virulent inoculum compared to mock (Fig. 2). AraCyc, RiceCyc, and MaizeCyc were able to detect different enriched metabolic pathways generated by Pathway Studio with 49 mapped metabolites out of 140 total metabolites differing between A195 and Sacramento.

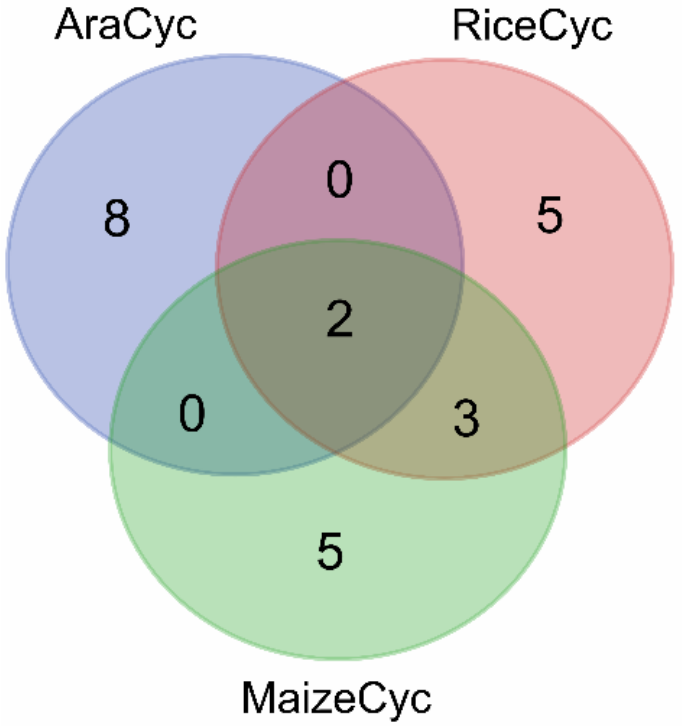

Figure 10: Venn Diagram showing overlapping pathways between AraCyc, RiceCyc, and MaizeCyc.

The top-10 ranked enriched cellular processes generated by GSEA in Pathway Studio with mapped metabolites differing between A195 and Sacramento were as follows: ripening, seed germination, nitrate uptake, plant growth, root growth, photosynthesis, Tricarboxylic acid cycle, translation, transmembrane potential, and breathing (Table 6). All cell processes listed are potential response differences between A195 and Sacramento in response to virulent inoculum.

Table 6: Top-10 ranked enriched cellular processes generated by GSEA in Pathway Studio with mapped metabolites differing between A195 and Sacramento.

Name	Total # of Neighbors	Overlap	Percent Overlap	Overlapping Entities	p-value†	Jaccard similarity§
Ripening	292	13	4	sucrose, L-asparagine, lipids, luteolin, L-phenylalanine, D-glucose, Phenolic acid, L-glutamate, palmitic acid, sitosterol, malate, citrate, L-aspartate	1.488E-11	4.08805E-2
Seed germination	1211	21	1	Phytol, sucrose, Nonanoate, isopentenyl-PP, L-asparagine, lipids, L-glycine, L-phenylalanine, urea, D-glucose, L-glutamine, stearic acid, L-tryptophan, nicotinate, L-glutamate, disaccharides, sitosterol, L-isoleucine, citrate, Nicotinamide, L-aspartate	9.08226E-11	1.70871E-2
Nitrate uptake	46	7	14	sucrose, L-glutamine, L-asparagine, L-glutamate, malate, urea, D-glucose	2.29688E-10	8.97436E-2
Plant growth	1000	19	1	sucrose, ethanolamine, lipids, luteolin, ferulic acid, L-glycine, urea, D-glucose, choline, L-glutamine, L-tryptophan, nicotinate, L-glutamate, allantoin, sitosterol, L-isoleucine, citrate, Nicotinamide, L-aspartate	2.52270E-10	1.86275E-2
Root growth	929	18	1	sucrose, ethanolamine, L-lactate, lipids, ferulic acid, urea, D-glucose, shikimic acid, choline, L-glutamine, L-tryptophan, nicotinate, L-glutamate, guanosine, palmitic acid, L-isoleucine, malate, citrate	6.91547E-10	1.89474E-2
Photosynthesis	604	15	2	sucrose, Phytol, lipids, L-glycine, urea, D-glucose, shikimic acid, choline, L-glutamine, L-tryptophan, Phenolic acid, L-glutamate, malate, citrate, L-aspartate	1.08680E-9	2.38854E-2
Tricarboxylic acid cycle	68	7	10	sucrose, L-lactate, lipids, malate, L-glycine, citrate, L-aspartate	3.92706E-9	7.00000E-2
Translation	245	10	4	sucrose, L-glutamine, L-tryptophan, L-asparagine, N-acetylglucosamine, L-glutamate, lipids, L-phenylalanine, D-glucose, L-aspartate	1.14257E-8	3.64964E-2
Transmembrane potential	92	7	7	sucrose, L-glutamine, L-glutamate, palmitic acid, L-glycine, malate, D-glucose	3.33224E-8	5.64516E-2
Breathing	218	9	4	sucrose, L-glutamate, palmitic acid, L-glycine, malate, citrate, urea, D-glucose, L-aspartate	6.25764E-8	3.62903E-2

One hundred and forty metabolites were found to vary between A195 and Sacramento relative to their mock controls. The metabolites included important metabolic classes such as amines, organic acids, sugars, flavonoids, phytoalexins, lipids, and fatty acids. Many of these metabolites are involved in important pathways associated with enhanced resistance.

Resistance to *S. sclerotiorum* is associated with variation in amines and amino acids at early time points

Amino acid abundance differed in A195 and Sacramento in response to virulent inoculum (Table 7). There was a decrease in abundance for many amino acids in A195 at 16 hpi with the exception of asparagine and n-acetylglucosamine. Asparagine and n-acetylglucosamine abundance increased in A195 post inoculation with virulent inoculum compared to mock. There was no decrease in amino acids in Sacramento and an increase in amino acids such as glycine and tryptophan post inoculation with virulent inoculum compared to mock (Fig. 3). A decrease in amino acids at 16 hpi in response to virulent inoculum in A195 suggests a mobilization of nitrogen as a resistance mechanism early during infection.

In general, amino acid variation diminished in A195 and Sacramento in response to virulent inoculum at 24 and 48 hpi. Pipecolic acid increased in A195 24 hpi with virulent inoculum compared to mock inoculum (Fig. 11). Pipecolic acid plays a significant role in systemic acquired resistance in plant immunity [57]. Abundances of pipecolic acid increased in A195 in response to virulent inoculum compared to mock inoculum, however, abundance in Sacramento also changed. Pipecolic acid has been found to accumulate in fungus-infected rice

leaves, and is considered an indicator of abnormal protein metabolism in diseased plants. It is also a metabolic defense signal that is essential for the establishment of systemic acquired resistance [57]. Therefore, pipecolic acid may be an important defense signal present in resistant lines of common bean when challenged with virulent inoculum. An increase in pipecolic acid highlights the importance of time as a variable in resistance to Sclerotinia; there is a delayed resistance response in A195, as there was no decrease in amino acids 48 hpi in both A195 and Sacramento in response to virulent inoculum. However, there was an increase in tryptophan, adenine, asparagine and glutamine in A195 48 hpi with virulent compared to mock inoculum. Amino acids differed between A195 and Sacramento in response to virulent compared to mock inoculum 24 and 48 hpi.

Table 7: Changes in amino acid and amine relative abundance at 0, 16, 24, and 48 hpi with virulent and mock inoculum in A195 and Sacramento represented as Studentized t-test, means ± se ABU, and z-scores.

Line		A195 vs Sacramento		A195				Sacramento			
Metabolite	**hpi**	**mock p-value †**	**inoc p-value †**	**inoc vs. mock p-value †**	**mean mock ± se ABU ‡**	**mean inoc ± se ABU‡**	**z-score ††**	**inoc vs. mock p-value †**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score ††**
adenine putative GC-MS	0	**< 0.01**									
	16	**0.01**	**0.04**	**0.3**	**52.98±2.04**	**37.66±4.74**	**-0.52**	**< 0.01**	**12.94±1.47**	**23.66±2.64**	**2.97**
	24	**0.04**	**0.36**	**< 0.01**	**23.88±5.88**	**83.36±7.58**	**5.06**	**0.01**	**15.9±3.14**	**33.52±4.87**	**2.29**
	48	**0.01**	**0.83**	**< 0.01**	**27.83±6.38**	**110.85±15.92**	**5.31**	**0.02**	**37.03±7.27**	**77.92±12.03**	**2.3**
alanine GC-MS	0	0.28									
	16	**< 0.01**	**0.35**	**< 0.01**	**2568.06±162.27**	**1667.24±168.68**	**-2.84**	**0.07**	**1455.55±137.3**	**1933.95±193.64**	**1.42**
	24	0.12	0.3	**0.01**	**1595.01±101.87**	**1201.88±65.19**	**-1.93**	0.72	1653.02±185.55	1733.31±106.73	0.18
	48	0.1	0.02	**0.03**	**1591.13±142.44**	**2012.4±102.21**	**1.21**	0.88	1901.85±169.88	1949.65±250.58	0.11
	0	0									

†† Z-score = [((mean of mock)-(mean of inoc))/(standard deviation of mock)]

aminoadipic acid GC-MS	16	**< 0.01**	0.93	0.12	47.52±3.39	27.6±7.95	-1.05	0.29	13.46±3.39	29.22±15.1	1.9
	24	0.83	0.14	0.26	27.45±6.015	20.63±2.36	-0.57	0.38	29.04±4.12	41.1±12.61	1.2
	48	0.87	0.08	**< 0.01**	**27.31±5.15**	**72.2±12.31**	**3.56**	0.3	25.32±10.71	41.86±9.59	0.63
amino-piperidone GC-MS	0	0.76									
	16	**< 0.01**	0.84	0.87	26.98±4.32	26.45±3.03	-0.26	**0.03**	**17.62±1.16**	**25.47±3.2**	**2.77**
	24	0.86	0.32	**0.05**	**59.37±19.78**	**23.81±1.91**	**-0.9**	0.13	96.63±47.1	18.45±1.98	-0.68
	48	0.56	0.22	0.41	125.5±22.18	95.07±30.63	-0.56	0.64	46.79±27.15	66.53±27.9	0.3
asparagine GC-MS	0	0.91									
	16	0.08	0.14	**0.02**	**1693.4±299.11**	**2714.63±367.75**	**4.61**	0.16	1019.6±332.2	1794.48±391.71	0.95
	24	0.16	0.35	0.21	3458.92±1061.48	2034.97±491.03	-0.67	0.27	1992.84±545.26	1287.27±266.86	-0.53
	48	**< 0.01**	0.26	**0.01**	**4061.1±434.41**	**6841.44±902.85**	**2.61**	0.21	2525.63±887.99	4047.82±582.83	0.7
aspartic acid GC-MS	0	0.93									
	16	**< 0.01**	0.97	**< 0.01**	**5733.04±247.39**	**4530.24±85.35**	**-5.24**	0.37	4094.68±230.66	4545.71±453.26	0.8
	24	0.13	0.71	0.41	4350.76±356.57	4006.52±226.21	-0.48	0.13	4625.21±272.74	4092.74±178.48	-0.8
	48	0.01	0.18	0.95	4829.91±113.29	4797.34±512.97	-0.12	0.19	4431.72±375.96	5059.29±140.93	0.68
choline LC-MS	0	**< 0.01**									
	16	**0.02**	0.31	0.72	5845.31±85.85	4991.18±595.38	-4.06	0.6	5066.17±269.66	4844.81±310.76	-0.34
	24	0.73	0.07	**0.01**	**5168.28±391.225**	**6520.02±172**	**1.73**	0.07	4270.04±155.21	4733.99±170.85	1.22
	48	0.64	0.52	**0.01**	**6301.33±170.42**	**5626.28±106.51**	**-1.62**	0.62	5014.9±331.59	4801.08±187.44	-0.26
	0	**< 0.01**									

ethanolamine GC-MS	16	0.73	0.86	**0.05**	**216.9±15.15**	**280.72±16.95**	**1.23**	0.24	226.16±15.93	290.91±53.8	1.66
	24	**0.04**	**0.01**	**0.04**	**282.94±50.365**	**458.74±46.56**	**1.75**	0.41	212.03±19.79	316.77±121.14	2.16
	48	**0.03**	0.3	0.45	371.42±36.73	335.24±30.32	-0.4	0.89	301.57±32.09	291.79±59.46	-0.12
glutamate GC-MS	0	0.73									
	16	0.96	**0.04**	0.18	1619.3±42.02	1291.71±197.94	-1.3	0.2	1629.36±168.46	1975.64±189.59	0.84
	24	0.16	0.25	**< 0.01**	**1920.62±282.695**	**768.41±61.05**	**-2.04**	0.77	1407.9±145.29	1461.78±104.29	0.15
	48	0.2	**0.03**	0.1	1940.77±90.9	2312.31±200.02	1.67	0.27	1711.87±268.78	2124.89±188.64	0.63
glutamate LC-MS	0	0.14									
	16	0.53	**0.03**	**< 0.01**	**868.24±47.91**	**575.23±52.7**	**-2.5**	0.69	809.92±76.61	862.37±104.87	0.28
	24	**0.05**	0.17	**0.02**	**670.11±18.515**	**377.68±59.55**	**-7.9**	0.79	699.94±42.53	721.74±67.88	0.21
	48	0.08	**< 0.01**	0.21	643.73±26.75	566.74±61.08	-1.18	0.57	793.2±71.23	727.23±79.95	-0.38
glutamine GC-MS	0	0.46									
	16	0.55	0.8	0.39	148.08±10.83	183.41±35.99	0.96	0.35	122.42±38.99	170.93±27.4	0.51
	24	0.82	**< 0.01**	**0.04**	**163.3±33.59**	**85.37±14.24**	**-1.16**	0.14	139.45±37.18	211.28±26.06	0.79
	48	0.19	0.82	**< 0.01**	**103.99±14.56**	**271.24±27.68**	**4.69**	0.25	100.89±8.11	167.77±53.78	3.37
glycine GC-MS	0	0.09									
	16	**< 0.01**	0.72	**< 0.01**	**394.37±8.03**	**243.34±27.16**	**-4.35**	**< 0.01**	**140.25±9.83**	**230.21±19.07**	**3.74**
	24	0.16	**0.05**	0.41	200.9±26.68	179.63±9.86	-0.4	**0.03**	**157.41±10.6**	**196.01±11.15**	**1.49**
	48	0.22	0.04	0.27	227.84±46.21	282.73±11.3	0.48	**0.01**	**140.27±14.71**	**221.13±19.26**	**2.24**
	0	0.1									

isoleucine GC-MS	16	**< 0.01**	0.71	**< 0.01**	**2182.13±7.5**	**1087.75±203.05**	**-6.09**	0.25	714.18±118.16	976.62±184.29	0.91
	24	0.68	**0.02**	**0.02**	**1655.63±55.125**	**1102.42±151.19**	**-5.02**	0.08	1194.38±156.2	847.84±81.54	-0.91
	48	0.16	0.4	0.09	2513.25±221.18	3000.99±156.61	0.9	0.69	1639.34±393.31	1428.1±275.02	-0.22
kynurenic acid LC-MS	0	0.08									
	16	0.83	0.11	**0.03**	**5.86±0.3**	**18.84±4.74**	**17.88**	0.2	6.08±0.92	8.16±1.25	0.92
	24	0.21	0.21	0.07	60.37±25.42	18.55±7.06	-0.82	0.62	6.44±1.13	5.71±0.89	-0.26
	48	0.15	**0.03**	0.1	9.67±1.32	33.33±15.42	7.34	0.21	8.54±1.38	12.9±2.85	1.29
leucine GC-MS	0	**0.02**									
	16	**< 0.01**	0.58	**< 0.01**	**2961.56±16.02**	**1289.66±274.37**	**-5.39**	0.33	1098.31±279.84	1550.18±340.49	0.66
	24	0.28	0.99	**< 0.01**	**2045.73±226.7**	**838.81±164.37**	**-2.66**	0.16	2057.25±478.34	1244.37±235.99	-0.69
	48	0.74	0.46	0.48	1813.71±403.38	2146.87±228.01	0.34	0.68	1952.95±513.92	1645.25±437.11	-0.24
leucine LC-MS	0	0.41									
	16	**< 0.01**	0.99	**< 0.01**	**2580.82±61.37**	**1158.05±196.03**	**-9.46**	0.51	876.45±158.79	1121.33±342.34	0.63
	24	0.09	0.38	**0.03**	**2150.85±435.28**	**987.33±214.55**	**-1.34**	0.21	1494.74±273.33	1046.21±197.94	-0.67
	48	0.12	**< 0.01**	0.9	2040.59±344.8	2087.31±137.59	0.06	0.27	2030.17±392.74	1415.18±296.97	-0.64
n-acetyl glucosamine GC-MS	0	**0.01**									
	16	0.06	0.83	**< 0.01**	35.92±0.72	45.05±0.75	4.11	0.53	41.31±2.38	44.16±3.87	0.49
	24	0.44	0.22	0.11	52.54±5.375	44.48±1.12	-0.75	1	37.19±1.17	37.17±2.2	0
	48	**0.02**	**0.03**	**0.03**	**57.35±3.45**	**47.41±1.69**	**-1.18**	0.42	35.04±2.39	38.32±2.9	0.56
	0	**< 0.01**									

nicotinamide GC-MS	16	0.15	0.99	0.07	30.82±1.39	36.07±1.73	1.21	**0.02**	**26.83±1.8**	**36.12±3.08**	**2.11**
	24	0.83	0.74	0.96	30.74±1.895	30.9±2.28	0.04	0.1	26.52±1.78	30.99±1.65	1.02
	48	**0.05**	0.63	**0.01**	**26.8±2.03**	**35.15±1.39**	**1.68**	0.15	27.31±0.88	30.27±1.64	1.37
ornithine GC-MS	0	0.86									
	16	**< 0.01**	0.66	0.18	116.13±32.46	91.48±16.26	-2.05	0.1	58.61±12.45	104.9±23.89	1.52
	24	0.54	0.09	0.06	336.36±141.625	89.72±12.33	-0.87	0.12	458.53±230.01	67.72±9.6	-0.69
	48	0.1	**0.01**	0.66	760.22±156.62	642.97±221.55	-0.31	0.64	267.13±187.03	404.35±200.38	0.3
pentasine LC-MS	0	0.03									
	16	**< 0.01**	0.27	**0.04**	**122.68±15.32**	**324.04±69.5**	**5.37**	**0.02**	**923.56±52.42**	**500.51±155.52**	**-3.29**
	24	0.44	0.23	0.07	116.77±8.025	158.88±11.11	2.62	0.57	1400.55±144.19	1234.36±246.43	-0.47
	48	**0.05**	0.06	0.79	162.27±15.37	168.92±23.3	0.18	0.79	700±164.79	767.01±159	0.17
phenylalanine GC-MS	0	0.95									
	16	**< 0.01**	0.72	**0.01**	**917.5±6.31**	**506.85±88.26**	**-1.96**	0.54	349.82±121.02	453.35±105.22	0.35
	24	0.66	0.39	0.08	684.71±62.33	455.6±81.11	-1.84	0.2	847.61±285.4	435.24±91.64	-0.59
	48	**0.03**	0.8	0.12	992.8±228.19	1453.13±155.39	0.82	0.32	644.76±275.32	1013.32±176.47	0.55
phenylalanine LC-MS	0	0.78									
	16	**< 0.01**	0.6	**< 0.01**	5231±417.47	2886.99±488.85	-2.29	0.69	1778.25±544.53	2146.55±717.12	0.28
	24	0.99	0.19	0.07	4072.96±961.3	2153.88±461.88	-1	0.24	4075.65±1250.65	2344.8±567.73	-0.56
	48	0.84	0.33	0.71	4826.2±1246.57	5357.68±664.68	0.17	0.49	3476.3±899.84	4282.11±519.43	0.37
	0	0.9									

pipecolic acid LC-MS	16	**< 0.01**	0.3	**< 0.01**	5243.72±651.49	1059.9±367.41	-2.62	0.41	738.79±81.63	831.95±65.24	0.47
	24	0.55	0.77	0.11	771.62±43.64	1297.98±195.63	6.03	0.99	709.37±56.27	710.17±43.16	0.01
	48	0.33	0.64	0.56	822.72±97.19	937.47±194.96	0.48	0.39	664.69±35.61	729.67±60.29	0.74
proline GC-MS	0	0.27									
	16	**< 0.01**	0.33	**< 0.01**	1699±13.05	368.08±83.23	-2.44	0.96	727.04±154.97	708.47±315.16	-0.05
	24	0.24	0.35	**0.03**	**836.55±351.555**	**118.34±23.97**	**-1.02**	0.2	1017.28±247.99	607.6±172.56	-0.67
	48	0.07	0.38	**0.03**	**1446.87±357.26**	**492.59±75.03**	**-1.09**	0.97	817.65±202.45	835.61±383.52	0.04
proline LC-MS	0	0.81									
	16	**< 0.01**	0.9	**0.01**	**1057.79±78.49**	**557.03±130.44**	**-2.6**	0.63	420.9±60.8	496.61±150.42	0.51
	24	0.23	0.07	**< 0.01**	**1173.75±254.485**	**501.02±54.97**	**-1.32**	0.08	1092.53±347.11	391.96±65.83	-0.82
	48	0.74	0.15	**0.05**	**1997.65±343**	**1163.74±204.64**	**-0.99**	0.9	913.06±211.74	963.33±290.82	0.1
serine GC-MS	0	0.24									
	16	**< 0.01**	0.95	**< 0.01**	**4161.25±246.06**	**2317.11±216.87**	**-7.16**	1	2347.1±304.14	2349.13±390.63	0
	24	0.11	**< 0.01**	**0.01**	**2752.39±231.59**	**1766.77±148.5**	**-2.13**	0.2	2932.85±378.03	2328.54±228.25	-0.65
	48	0.44	0.51	0.62	2977.58±289.14	3162.59±246.67	0.26	0.12	2792.25±256.7	3372.96±189.61	0.92
threonine GC-MS	0	0.53									
	16	**< 0.01**	0.53	**< 0.01**	**1005.95±6.19**	**484.17±56.13**	**-7.21**	0.39	458.04±62.45	553.18±86.5	0.62
	24	0.7	0.19	**0.01**	**642.69±35.125**	**395.95±52.97**	**-3.51**	**0.05**	**621.66±74.64**	**420.47±47.24**	**-1.1**
	48	0.07	0.45	**< 0.01**	**789.66±78.41**	**477.37±33.89**	**-1.63**	0.24	560.04±67.65	446.99±51.65	-0.68
	0	**0.02**									

tryptophan GC-MS	16	**< 0.01**	0.42	0.92	298.79±4.75	289.41±86.31	-0.16	0.07	119.13±26.83	204.86±33.56	1.3
	24	**< 0.01**	0.02	0.42	343.99±53.305	290.68±37	-0.5	0.43	214.06±53.83	269.48±39.52	0.42
	48	**< 0.01**	0.21	**< 0.01**	**504.59±99.16**	**1365.84±168.66**	**3.55**	0.19	331.1±73.46	517.15±103.96	1.03
tryptophan LC-MS	0	0.26									
	16	**< 0.01**	0.37	0.65	3100.37±343.1	2996.8±493.46	-0.12	**0.03**	**903.63±177.71**	**2127.78±485.43**	**2.81**
	24	0.45	0.32	0.06	4324.36±1083.1	2238.23±481.43	-0.96	0.77	2283.63±557.09	2490.89±411.63	0.15
	48	0.37	0.03	0.1	3879.1±374.52	6409.17±1521.31	2.76	0.34	2892.39±809.87	4048.07±717.41	0.58
tyrosine LC-MS	0	0.88									
	16	**< 0.01**	0.5	**< 0.01**	**871.16±63.67**	**263.08±28.48**	**-3.9**	0.78	350.3±97.34	309.4±98.36	-0.17
	24	0.54	0.2	**0.01**	**1109.72±335.81**	**97.01±15.11**	**-1.51**	0.09	588.26±140.68	246.86±116.32	-0.99
	48	0.77	0.3	**0.01**	**652.03±155.85**	**174.59±16.18**	**-1.25**	**0.05**	**465.19±107.86**	**183.61±36.73**	**-1.07**
valine GC-MS	0	0									
	16	**< 0.01**	0.69	**< 0.01**	**3180.58±13**	**1640.12±304.58**	**-5.49**	0.32	1149.18±168.54	1465.81±261.21	0.77
	24	0.66	0.87	**0.01**	**2396.86±75.67**	**1494.82±227.4**	**-5.96**	**0.05**	**1823.34±235.78**	**1269.44±96.87**	**-0.96**
	48	0.35	0.09	0.38	3184.19±297.23	3490.73±164.32	0.42	0.76	2200.38±497.13	2000.19±337.29	-0.16
valine LC-MS	0	0.15									
	16	**< 0.01**	0.93	**< 0.01**	**785.16±35.29**	**309.24±67.57**	**-5.4**	0.47	228.03±47.41	298.89±87.06	0.61
	24	**< 0.01**	0.84	0.19	435.94±61.085	285.27±62.28	-1.23	0.27	383.75±52.26	292.55±57.77	-0.71
	48	**< 0.01**	**< 0.01**	0.81	617.46±125.52	584.5±43.33	-0.11	0.26	524.89±60.01	393.71±87.49	-0.89

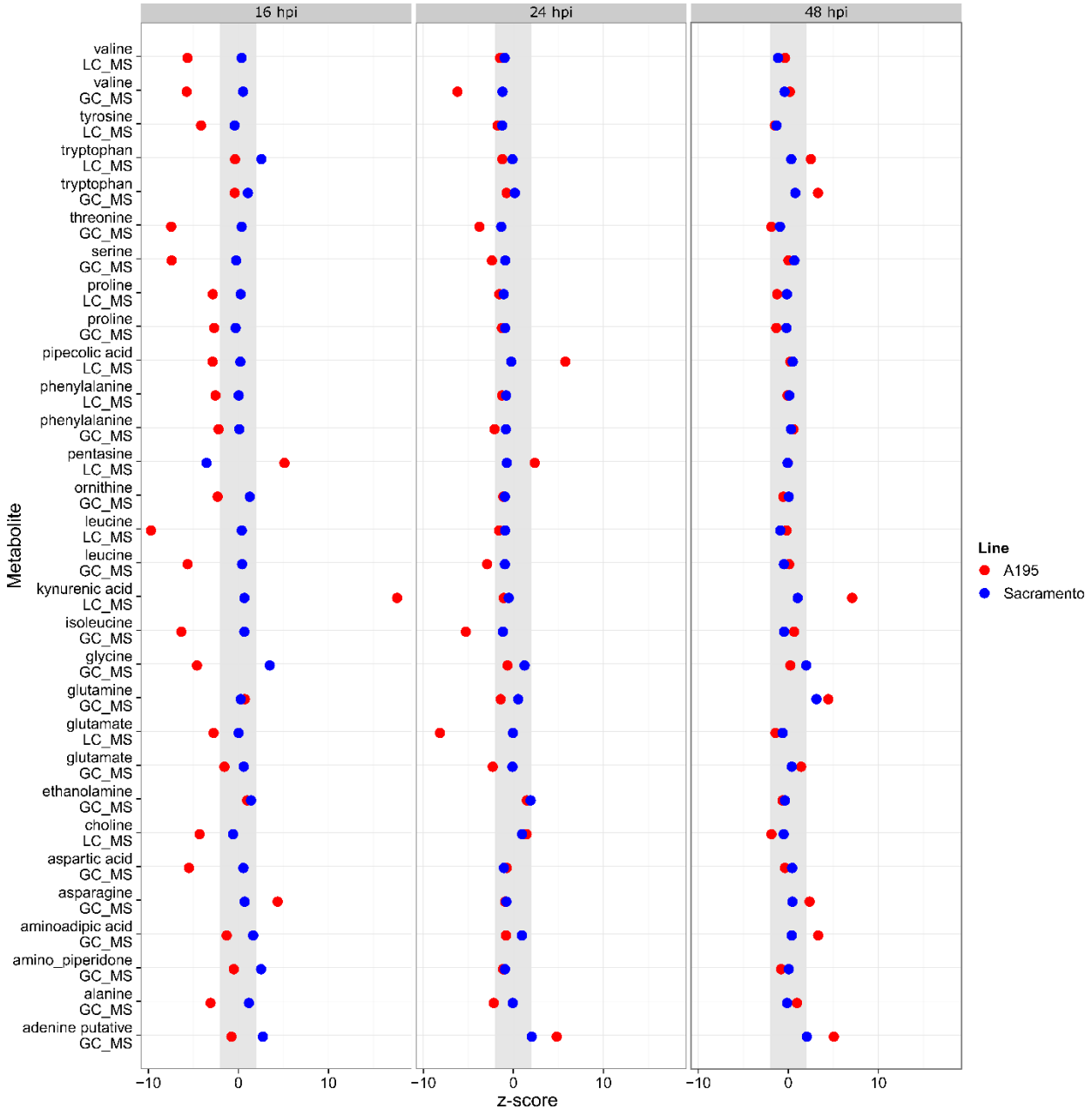

Figure 11: Z scores that illustrate the relative changes in amines/amino acid abundance at 16, 24, and 48 hpi in A195 and Sacramento when challenged with virulent inoculum relative to mock inoculum. Grey regions indicate areas of insignificant data points.

Resistance to *S. sclerotiorum* is associated with increased ureides at early time points and may be a mechanisms of nitrogen remobilization

Ureides differed in abundance between A195 and Sacramento in response to virulent inoculum compared to mock inoculum (Table 8). Ureides had the greatest change in response to virulent inoculum in the overall metabolome for A195. Ureides did not change in Sacramento in response to virulent inoculum compared to mock. Urea and allantoin increased in A195 at 16 hpi ($p < 0.01$) with virulent inoculum compared to mock (Fig. 12). Guanosine, a purine precursor to ureide synthesis decreased in A195 at 16 hpi with virulent inoculum compared to mock [58]. There was an increased abundance of urea and allantoin and a decreased abundance of guanosine in A195 compared to Sacramento in response to virulent inoculum. Degradation of guanosine to produce urea and allantoin may be a resistance mechanism in A195 by providing a means for nitrogen remobilization away from the site of infection.

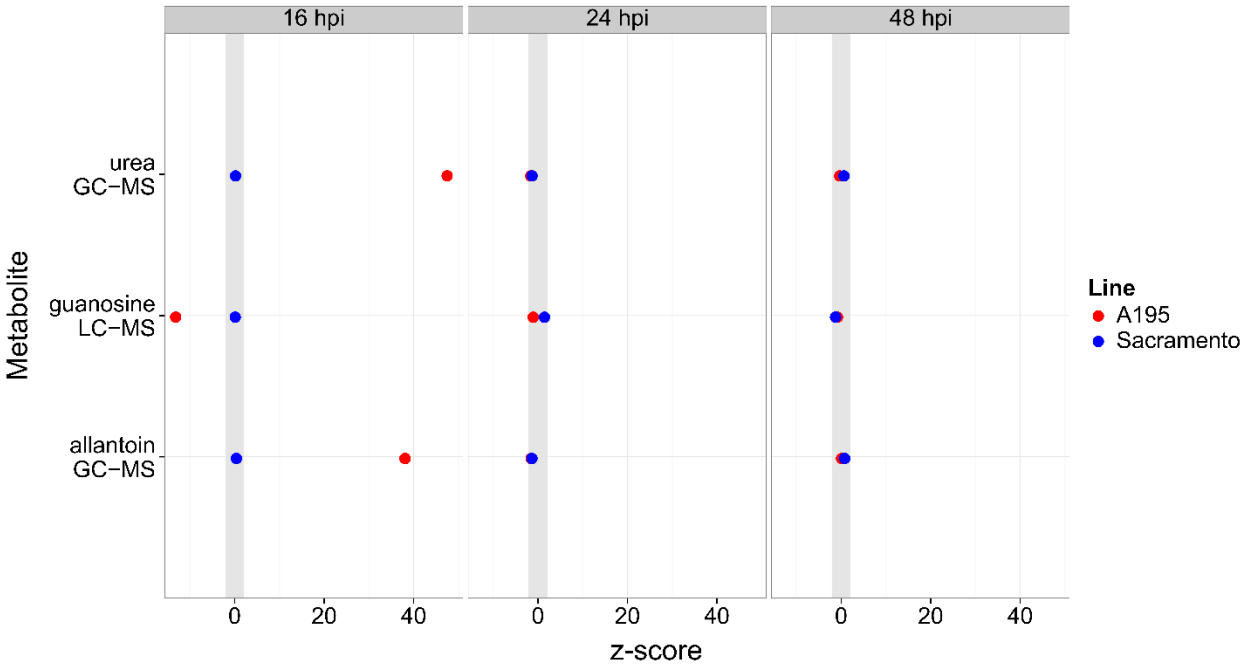

Figure 12 Z scores that illustrate the relative changes in ureide abundance at 16, 24, and 48 hpi in A195 and Sacramento when challenged with virulent inoculum relative to mock inoculum. Gray regions indicate areas of insignificant data points ($p > 0.05$).

Table 8: Changes in ureides relative abundance at 0, 16, 24, and 48 hpi with virulent and mock inoculum in A195 and Sacramento represented as Studentized t-test, means ± se ABU, and z-scores.

		A195 vs Sacramento		A195				Sacramento			
Metabolite	**hpi**	**mock †**	**inoc †**	**inoc vs. mock †**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score ††**	**inoc vs. mock †**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score ††**
guanosine LC-MS	0	0.22									
	16	**< 0.01**	0.6	**< 0.01**	**143.38±3.16**	**45.73±10.1**	**-12.62**	0.47	49.94±9.81	66.32±20.74	0.68
	24	0.38	0.08	0.1	68.36±23.86	44.85±5.52	-0.49	**0.02**	**65.51±12.21**	**126.22±16.76**	**2.03**
	48	**0.02**	0.35	0.59	77.01±18.89	64.69±12.2	-0.27	0.18	144.61±35.01	84.22±15.71	-0.7
urea GC-MS	0	0.68									
	16	0.16	0.39	**0.01**	**88.64±49.03**	**362.41±82.45**	**48.04**	0.25	168.25±51.94	264.4±58.73	0.76
	24	0.1	0.29	**0.03**	**452.91±154.065**	**132.25±20.31**	**-1.04**	0.1	362±137.98	106.26±18.76	-0.76
	48	0.95	0.74	0.74	604.49±150.24	685.27±194.77	0.22	0.2	200.02±71.82	407.86±129.08	1.18
allantoin GC-MS	0	0.63									
	16	0.16	0.61	**0.01**	**54.95±37.81**	**396.19±104.49**	**38.62**	0.24	155.63±66.62	311.43±112.23	0.95
	24	0.48	0.57	**0.05**	**499.23±193.535**	**127.76±30.13**	**-0.96**	0.09	328.88±135.6	74.07±13.79	-0.77
	48	0.26	**0.02**	0.36	478.97±86.71	612.4±117.41	0.63	0.11	195.57±52.25	367.63±79.49	1.34

The mobilization and recycling of nitrogen is a well-documented defense strategy in many plants during the course of pathogen invasion [59]. There was a general decrease in amino acid abundances at 16 hpi with virulent inoculum. Among the amino acids detected in A195, only asparagine increased in leaf tissue, the remaining either did not change or decreased in abundance. Asparagine is normally transported from infected cells to the xylem and distributed to healthy parts of the plant. Guanosine decreased in A195 in response to virulent inoculum at 16 hpi compared to mock. However, it increased in response to virulent inoculum in Sacramento at 24 hpi. Purines such as adenine and guanosine are precursors to synthesize uric acid. During remobilization events, uric acid is transported from infected cells to uninfected cells and acts as a precursor for the production of allantoin and allantoate, which can then be shuttled through the xylem to healthy uninfected tissue (Fig. 13) [58]. The pathway involving uric acid produces hydrogen peroxide as a by-product as it is converted to allantoin [60]. Allantoin increased in A195 in response to virulent inoculum compared to mock, however, it did not change in Sacramento. In common bean leaves infected with anthracnose [*Colletotrichum lindemuthianum* ((Sacc. & Magnus) Briosi & Cavara], the mobilization of nitrogenous compounds is believed to be a “slash-and-burn” approach that deprives the pathogen of nutrients [61]. Allantoate can be degraded to the by-product urea which also increased in A195 in response to virulent inoculum. However, urea did not change in Sacramento in the same treatment. These results suggest that nitrogen remobilization is a potential mechanism to enhance resistance in common bean to deprive the pathogen of important nitrogenous nutrients.

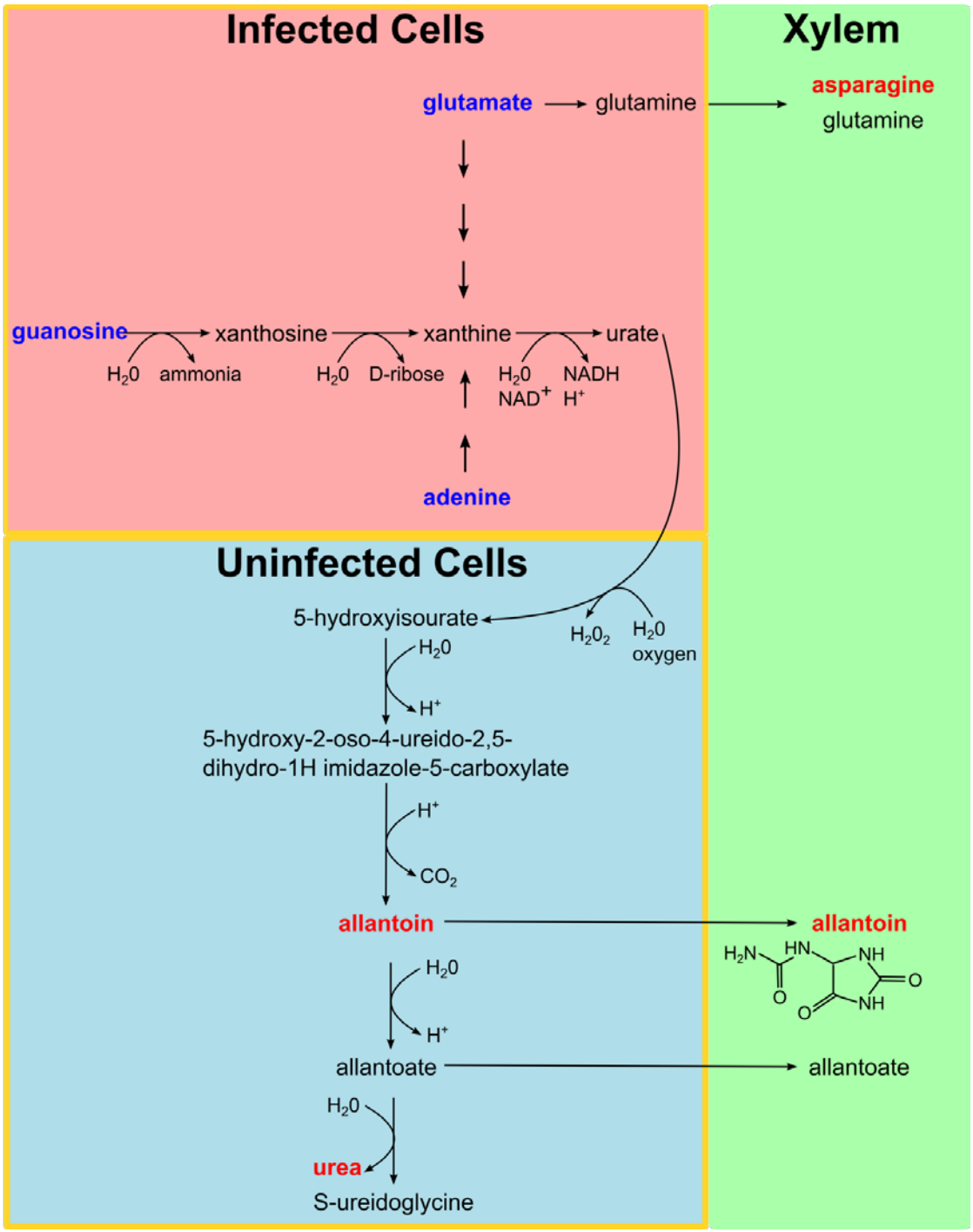

Figure 13: Nitrogenous compounds shuttled away from infected and uninfected cells [47, 59]. A large amount of the fixed nitrogen is exported as uric acid, a decomposition product of purines, to adjacent uninfected cells. Uric acid is degraded to allantoin and allantoic acid in uninfected cells which are then transported through the xylem to the rest of the plant. Red text are metabolites found to be increased in A195. Blue text are metabolites found to be downregulated in A195.

Resistance to *S. sclerotiorum* is associated with variation in organic acids at early time points

Organic acids differed in abundance between A195 and Sacramento in response to virulent vs mock inoculum (Table 9). Most organic acids increased in abundance in A195 at 16 hpi with virulent compared to mock inoculum. Organic acids that increased in abundance included shikimic acid, xanthurenic acid, nicotinic acid, malic acid, maleic acid, and imidazolidone carboxylic acid. There were minimal changes in organic acids in Sacramento at 16 hpi with virulent inoculum compared to mock. More metabolites responded to virulent inoculum in A195 compared to Sacramento 16 hpi (Fig. 14).

There was minimal change in organic acids in A195 and Sacramento at 24 hpi with virulent inoculum compared to mock. There was no change in organic acids in Sacramento at 24 hpi with virulent inoculum compared to mock. There was an increase in succinate and shikimate acid in A195 at 48 hpi with virulent compared to mock, which was not seen at 24 hpi. Hydroxymethyglutaric acid and isopropylmalic acid increased in both A195 and Sacramento at 48 hpi with virulent compared to mock inoculum, which was not seen at 16 or 24 hpi (Fig. 14). Organic acids differed in A195 and Sacramento in response to virulent inoculum compared to mock inoculum. These results suggest that the resistance mechanism associated with organic acids in A195 happens early on at 16 hpi rather than later at 24 hpi or 48 hpi.

Table 9: Changes in organic acid relative abundance at 0, 16, 24, and 48 hpi with virulent and mock inoculum in A195 and Sacramento represented as Studentized t-test, means ± se ABU, and z-scores.

		A195 vs Sacramento		A195				Sacramento			
Metabolite	**hpi**	**mock †**	**inoc†**	**inoc vs. mock †**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score ††**	**inoc vs. mock †**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score††**
2-caffeoylisocitrate, putative LC-MS	0	0.23									
	16	0.09	0.9	**< 0.01**	**12.71±0.44**	**8.97±0.69**	**-3.48**	0.71	10.09±1.33	9.23±1.82	-0.26
	24	0.87	**0.01**	**0.02**	**23.49±6.135**	**6.45±0.49**	**-1.39**	0.53	11.18±1.58	9.74±1.58	-0.37
	48	0.12	0.96	0.08	15.05±2.18	21.27±2.85	1.17	0.44	14.09±2.47	11.71±1.21	-0.39
citric acid GC-MS	0	0.17									
	16	0.22	0.13	0.18	2147.09±551.56	2985.88±562.44	2.85	0.07	2835.93±509.5	4538.91±682.56	1.36
	24	0.25	0.04	**0.04**	**3025.85±461.66**	**1783.97±262.74**	**-1.35**	0.5	2409.03±287.7	2792.6±460.03	0.54
	48	0.11	0.86	**0.02**	**2305.21±368.02**	**4124.28±584.24**	**2.02**	0.13	2376.39±372.64	3665.53±658.75	1.41
dihydroxymaloni c-acid GC-MS	0	0.24									
	16	**< 0.01**	0.73	**0.01**	**316.12±19.3**	**194.8±15.91**	**-1.77**	0.16	139.2±12.48	214.44±52.62	2.46
	24	**< 0.01**	**0.02**	0.5	205.44±13.61	276.49±79.42	2.61	0.9	177.31±47.81	187.1±60.19	0.08
	48	**0.04**	**0.03**	0.18	89.67±27.72	178.32±60.92	1.31	0.91	192.08±31.04	199.59±52.01	0.1
ferulic acid GC-MS	0	**0.01**									
	16	**0.01**	**< 0.01**	0.77	21.23±1.55	21.89±2.09	0.36	0.81	34.2±3.85	35.27±0.9	0.11
	24	0.09	0.1	**0.02**	**34.29±6.445**	**18.57±1.06**	**-1.22**	0.46	32.04±4.04	27.34±4.52	-0.48
	48	0.99	0.1	**< 0.01**	27.54±1.97	41.65±2.76	2.92	0.72	30.26±4.33	32.62±4.08	0.22
ferulic acid LC-MS	0	**0.02**									
	16	**< 0.01**	**< 0.01**	**< 0.01**	**814.16±22.74**	**1060.59±57.93**	**4.42**	0.32	1405.37±148.03	1580.22±34.19	0.48
	24	0.79	0.07	0.13	1157.37±264.035	740.28±60.13	-0.79	0.98	1288.78±190.01	1297.41±265.38	0.02
	48	0.25	0.68	0.29	1026.72±92.04	1141.97±52.54	0.51	0.75	1352.55±207.94	1273.45±84.17	-0.16
hydroxybenzoic acid GC-MS	0	**0.05**									
	16	0.11	0.95	0.32	69.68±7.55	75.71±3.48	0.59	**0.01**	**61.97±1.46**	**75.33±4.44**	**3.74**
	24	0.1	0.83	**0.03**	**73.4±5.01**	**93.41±5.41**	**2**	0.97	70.84±6.02	70.31±11.59	-0.04
	48	0.25	0.27	0.98	90.54±7.94	90.3±6.82	-0.01	0.37	71.95±8.85	84.14±8.53	0.56
	0	**< 0.01**									

Hydroxymethyl-glutaric acid GC-MS	16	**< 0.01**	**0.01**	0.79	5.11±0.1	5.29±0.66	0.59	0.23	2.37±0.21	2.73±0.17	0.69
	24	0.18	0.53	0.36	7.58±1.495	6.25±0.55	-0.45	0.13	3.09±0.31	3.8±0.29	0.94
	48	0.07	0.83	**< 0.01**	**7.01±0.67**	**16.37±2.25**	**5.72**	**< 0.01**	**4.14±0.52**	**7.98±0.71**	**2.99**
imidazolidone carboxylic acid GC-MS	0	0.85									
	16	0.35	**0.02**	**< 0.01**	**124.61±28.13**	**346.16±32.04**	**18.99**	0.05	96.52±28.18	203.03±38.33	1.54
	24	0.11	0.36	0.51	350.09±111.32	268.84±64.27	-0.36	0.21	314.41±128.34	138.14±32.03	-0.56
	48	0.37	0.17	0.35	504.29±26.64	612.52±118.14	1.66	0.32	269.39±119.39	450.62±109.62	0.62
isopropylmalic acid GC-MS	0	**0.02**									
	16	0.1	0.33	0.1	5.82±1.17	4.28±0.76	-1.93	0.25	4.39±0.71	3.37±0.3	-0.58
	24	0.24	0.4	0.41	4.73±0.575	3.99±0.58	-0.65	**< 0.01**	**5.32±0.64**	**2.82±0.24**	**-1.6**
	48	0.44	0.3	**< 0.01**	**5.36±0.98**	**19.9±4.1**	**6.07**	**0.05**	**3.36±0.48**	**5.38±0.71**	**1.72**
lactate GC-MS	0	0.63									
	16	0.47	0.63	0.09	639.32±399.9	1400.64±311.33	1.38	0.06	453.74±95.19	1159.07±346.17	3.03
	24	0.88	0.17	0.23	730.26±275.475	434.57±49.12	-0.54	0.8	805.8±220.14	878.11±168.85	0.13
	48	0.29	0.63	0.08	358.82±81.96	186.26±34.48	-0.86	0.89	285.3±47.85	274.82±49.08	-0.09
maleic acid GC-MS	0	**0.01**									
	16	**0.01**	0.51	**0.01**	**1729.74±275.82**	**3045.36±365.56**	**8.71**	0.25	2747.48±317.8	3541.1±605.2	1.02
	24	0.85	0.1	0.2	2047.8±260.915	2784.03±386.74	1.41	0.26	2664.94±350.37	3223.18±313.3	0.65
	48	0.08	0.34	**< 0.01**	**2291.38±193.88**	**3375.74±225.33**	**2.28**	**< 0.01**	**2062.68±205.75**	**3086.56±136.48**	**2.03**
malic acid GC-MS	0	**0.05**									
	16	0.46	0.35	**0.05**	**3263.9±289.91**	**4464.37±513.03**	**4.74**	0.78	3553.68±366.55	3730.08±493.04	0.2
	24	0.45	0.38	**0.2**	**4266.79±612.585**	**3222.04±457.05**	**-0.85**	0.68	3738.69±340.05	3930.38±303.07	0.23
	48	0.1	0.1	**0.01**	**3446.5±285.68**	**4641.98±240.14**	**1.71**	0.3	4204.74±179.65	3810.5±300.14	-0.9
nicotinic acid GC-MS	0	**0.02**									
	16	0.13	0.36	**0.02**	**56.34±16.52**	**85.35±10.06**	**4.73**	0.35	66.36±5.53	73.71±4.77	0.54
	24	0.51	0.44	**0.02**	**61.63±14.76**	**110.42±10.29**	**1.65**	0.77	61.58±1.88	58.82±8.82	-0.6
	48	0.1	0.65	0.06	116.75±15.95	76.69±11.1	-1.03	0.12	55.06±5.37	70.49±6.75	1.17
nonanoic acid GC-MS	0	0.06									
	16	0.12	**0.03**	**0.01**	**34.71±5.17**	**47.08±2.55**	**2.02**	0.22	41.68±3.28	35.55±3.24	-0.76
	24	0.48	0.14	0.56	41.72±5.545	45.56±3.66	0.35	0.98	37.75±2.91	37.65±4.7	-0.02
	48	0.02	0.12	0.77	49.14±5.47	46.99±5.26	-0.16	0.59	38.29±3.15	35.68±3.07	-0.34
orotic acid, putative GC-MS	0	0.96									
	16	**0.02**	0.45	**< 0.01**	**5.58±0.73**	**10.08±0.87**	**8.09**	**< 0.01**	**7.5±0.63**	**10.94±0.52**	**2.23**
	24	0.62	0.98	0.8	7.19±0.42	6.98±0.58	-0.25	0.35	10.09±1.28	8.31±1.27	-0.57

	48	0.21	0.31	0.06	7.04±1.02	9.92±1.02	1.15	0.34	9.43±2.36	12.28±1.19	0.49
phytol GC-MS	0	0.69									
	16	**< 0.01**	0.34	**< 0.01**	**49.94±2.43**	**81.76±4.61**	**7.29**	**0.04**	**59.3±0.87**	**73.46±6.42**	**6.6**
	24	0.45	0.33	0.2	72.37±8.43	102.12±16.25	1.76	0.38	58.84±1.91	67.63±9.36	1.88
	48	0.54	0.19	0.08	70.3±3.08	124.37±30.64	7.17	0.71	70.45±8.23	66.04±7.11	-0.22
shikimic acid GC-MS	0	**0.03**									
	16	0.07	0.22	0.27	132.03±68.98	177.91±38.14	2.49	0.43	92.07±18.23	115.33±21.56	0.52
	24	0.92	0.12	0.36	77.65±16.69	103.01±17.78	0.76	0.13	99.65±15.08	147.68±25.26	1.3
	48	0.56	0.12	**0.01**	**51.31±3.93**	**100.8±14.86**	**5.14**	0.12	56.22±10.83	100.03±23.16	1.65
succinate GC-MS	0	**0.02**									
	16	**0.01**	0.38	**0.01**	**53.09±22.1**	**135.18±23.02**	**6.17**	0.2	118.8±21.42	178.72±39.91	1.14
	24	0.12	**< 0.01**	0.15	58.81±14.02	116.97±27.79	2.07	0.31	120.88±25.42	159.06±25.14	0.61
	48	0.47	0.29	**< 0.01**	**63.51±9.9**	**161.04±20.59**	**4.02**	**< 0.01**	**64.66±13.86**	**153.3±9.52**	**2.61**
succinate LC-MS	0	**0.01**									
	16	0.08	0.68	**< 0.01**	**35.64±3.56**	**81.61±9.46**	**5.27**	0.97	79.87±22.69	80.77±9.89	0.02
	24	0.21	0.84	0.28	47.26±13.99	82.25±16.61	1.25	0.17	76.52±17.09	111.1±16.4	0.83
	48	0.98	0.07	**< 0.01**	**44.03±2.65**	**87.87±7.24**	**6.75**	**0.01**	**54.97±6.31**	**88.51±8.59**	**2.17**
toluic acid, putative GC-MS	0	0.17									
	16	0.19	0.19	**< 0.01**	**82.22±9.04**	**135.68±6.87**	**4.34**	0.38	93.61±6.23	108.97±16.95	1.01
	24	0.68	0.37	**0.03**	**96.09±5.94**	**131.24±9.94**	**2.96**	0.39	93.77±5	103.95±10.08	0.83
	48	**0.04**	0.27	0.21	113.59±11.4	96.65±5.96	-0.61	0.59	94.04±5.68	101.17±11.22	0.51
xanthurenic acid, putative GC-MS	0	0.1									
	16	0.34	**0.05**	**0**	**1501.14±247.86**	**2930.92±142.69**	**3.48**	0.3	1738.54±166.87	2110.31±309.28	0.91
	24	0.91	**< 0.01**	0.11	1882.04±204.6	2462.56±220.23	1.42	0.67	1853.52±104.36	1928.18±131.02	0.29
	48	0.12	0.35	0.22	2172.27±271.11	1773.75±148.86	-0.6	0.53	1743.82±139.36	1912.9±209.1	0.5

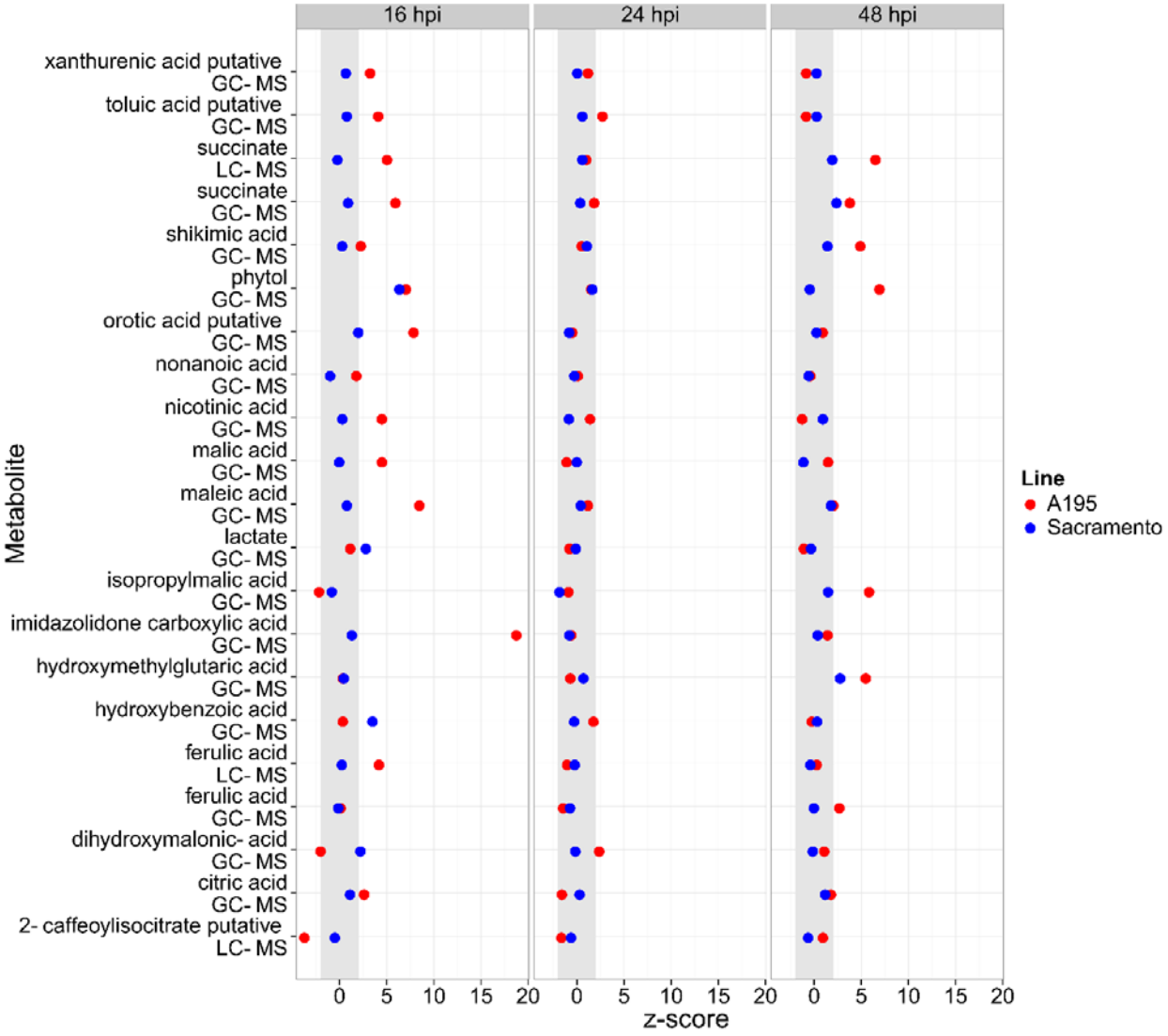

Figure 14: Z scores that illustrate the relative changes in organic acid abundance at 16, 24, and 48 hpi in A195 and Sacramento when challenged with virulent inoculum relative to mock inoculum. Gray regions indicate areas of insignificant data points ($p > 0.05$).

Resistance to *S. sclerotiorum* is not associated with saccharides

Sugars differed in abundance between A195 and Sacramento in response to virulent inoculum (Table 10). There were both increased and decreased sugars in A195 in response to virulent inoculum at 16 hpi compared to mock. Sugars gradually increased in response to virulent inoculum compared to mock in A195 at 24 and 48 hpi. The majority of sugars in Sacramento did not respond to virulent inoculum at 16, 24, or 48 hpi compared to mock (Fig. 15). Sugars changed more in A195 than Sacramento in the presence of virulent inoculum. A change in the primary metabolism of sugars may be involved in resistant mechanisms in A195.

Table 10: Changes in sugar relative abundance at 0, 16, 24, and 48 hpi with virulent and mock inoculum in A195 and Sacramento represented as Studentized t-test, means ± se ABU, and z-scores.

		A195 vs Sacramento		A195				Sacramento			
Metabolite	**hpi**	**mock †**	**inoc†**	**inoc vs. mock †**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score ††**	**inoc vs. mock †**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score ††**
c5 hexose GC-MS	0	0.2									
	16	0.17	0.93	**< 0.01**	**284.03±24.19**	**230.92±6.44**	**-2.75**	0.11	264.93±10.33	229.05±18.58	-1.42
	24	0.23	**0.01**	0.22	280.66±30.1	240.15±15.5	-0.67	0.39	236.38±12.59	221.4±10.87	-0.49
	48	0.87	**0.02**	0.9	307.87±14.41	302.57±40.07	-0.15	0.08	207.13±14.63	250.62±14.8	1.21
c5 sugar acid GC-MS	0	**0.04**									
	16	**0.02**	0.14	**< 0.01**	**60.25±3.94**	**41.24±2.63**	**-2.61**	0.58	47.95±3.38	51.46±5.47	0.42
	24	0.43	0.17	0.1	53.87±3.515	43.29±4.02	-1.5	0.77	46.64±2.57	45.35±3.53	-0.21
	48	**0.02**	0.42	0.34	63.54±3.89	71.91±8.09	0.88	**0.04**	**44.98±4.41**	**58.96±3.41**	**1.29**
c6 sugar GC-MS	0	0.54									
	16	**< 0.01**	0.61	**0.05**	**5128.41±161.65**	**3194.6±858.44**	**-17.64**	**< 0.01**	**4691.96±70.05**	**2637.53±495.42**	**-11.97**
	24	0.09	0.18	0.98	3568.76±498.385	3588.26±471.39	0.02	0.98	4034.08±397.78	4054.9±649.3	0.02
	48	0.25	0.25	0.16	2534.94±677.7	1371.38±412.98	-0.7	0.45	3491.53±420	3005.16±413.87	-0.47
	0	0.45									

c6 sugar GC-MS	16	**0.01**	0.65	**< 0.01**	**78.52±3.3**	**45.19±5.54**	**-2.48**	**0.05**	**57.05±3.28**	**40.73±7.08**	**-2.03**
	24	0.45	**0.01**	0.24	60.19±6.175	49.36±5.59	-0.88	0.82	50.98±5.1	49.24±5.5	-0.14
	48	0.16	0.3	0.16	52.77±9.43	35.92±6.32	-0.73	0.2	56.79±7.21	42.82±6.36	-0.79
c6 sugar pyranose GC-MS	0	0.2									
	16	0.06	0.71	**0.02**	**1565.75±53.62**	**848.74±243.37**	**-2.96**	**0.01**	**1303.68±76.41**	**723.62±181.59**	**-3.1**
	24	0.87	0.23	0.95	807.88±187.03	821.7±128.7	0.04	0.76	1149.81±133.47	1081.86±170.9	-0.21
	48	**0.02**	0.57	0.18	521.97±129.32	307.61±77.82	-0.68	0.96	773.22±127.96	762.42±154.06	-0.03
cyclic sugar alcohol GC-MS	0	**0.02**									
	16	**0.04**	0.79	**< 0.01**	**158.93±13.4**	**109.14±11.87**	**-4.52**	0.37	128.45±12.49	113.45±8.91	-0.49
	24	0.6	**< 0.01**	**< 0.01**	**235.05±27.32**	**102.4±11.19**	**-2.43**	0.12	129.96±10.74	105.49±9.29	-0.93
	48	0.08	0.13	**0.01**	**168.74±8.64**	**225.65±18.79**	**2.69**	0.45	106.26±14.96	125±17.16	0.51
disaccharide GC-MS	0	0.68									
	16	0.14	0.7	0.13	325.85±41.26	437.96±67.41	3.97	0.09	357.54±16.04	477.35±67.77	3.05
	24	0.4	0.07	**0.03**	**588.11±124.425**	**304.59±37.94**	**-1.14**	0.51	303.1±11.52	317.68±18.06	0.52
	48	0.22	0.26	**0.01**	**272.19±35.57**	**560.06±92.94**	**3.3**	0.71	363.47±76.83	409.24±85.41	0.24
dissaccharide GC-MS	0	0.08									
	16	0.29	0.08	**0.05**	**1263.98±95.42**	**874.97±75.76**	**-1.13**	0.2	1610.88±276.03	1160.01±114.36	-0.67
	24	**0.02**	0.11	0.08	800.21±132.375	1116.92±92.18	1.2	0.65	1972.33±205.02	2172.26±378.96	0.4
	48	0.93	0.21	**0.03**	**843.15±82.85**	**1235.66±145.7**	**1.93**	0.57	1990.78±278.39	1728.08±332.87	-0.39

erythronic acid lactone GC-MS	0	0.08									
	16	**0.04**	**0.05**	0.08	14.45±0.67	11.31±0.78	-1.01	**0.02**	**18.56±1.16**	**14.35±0.98**	**-1.48**
	24	**< 0.01**	**< 0.01**	0.14	12.76±1.145	9.65±1.31	-1.36	**< 0.01**	**17.79±0.85**	**12.02±0.79**	**-2.78**
	48	**< 0.01**	**0.01**	**0.01**	**17.56±1.42**	**11.79±0.99**	**-1.66**	0.94	12.13±1.41	12.26±0.91	0.04
fructose GC-MS	0	**< 0.01**									
	16	0.25	0.52	**< 0.01**	**13617.2±742.24**	**7258.69±1567.44**	**-9.8**	0.11	12442.83±924.66	8963.64±1880.52	-1.54
	24	1	0.8	0.61	6750.18±3233.065	8595.73±1841.41	0.29	0.44	9880.64±2338.44	12525.61±2328.29	0.46
	48	0.4	0.24	0.14	4408.76±1165.36	2180.44±830.86	-0.78	0.16	11063.65±2009.32	6909.95±1631.44	-0.84
glucopyranose GC-MS	0	0.19									
	16	**0.02**	**< 0.01**	**0.01**	**393.42±11.64**	**706.9±100.16**	**7.82**	0.44	235.41±52.47	187.2±15.97	-0.38
	24	0.22	0.7	0.07	456.3±98.29	797.24±112.64	1.73	**0.01**	**298.37±59.11**	**694±110.1**	**2.73**
	48	0.09	0.56	0.08	392.19±82.38	830.84±233.14	2.17	**< 0.01**	**212.38±27.28**	**885.26±54.77**	**10.07**
glucose GC-MS	0	0.34									
	16	0.42	0.47	0.2	11670.51±197.95	9722.5±1400.83	-4.71	**< 0.01**	**11359.33±330.57**	**8461.6±619.31**	**-3.58**
	24	0.1	0.7	0.2	9435.22±829.335	10978.65±717.47	0.93	0.63	10444.32±859.24	9856.36±808.4	-0.28
	48	0.29	0.18	0.15	7880.74±1489.49	5044.81±1180.49	-0.78	0.57	9400.97±666.49	8781.25±762.52	-0.38
sedoheptulose GC-MS	0	0.24									
	16	0.14	0.04	0.46	262.93±23.39	250.45±13.23	-0.6	0.94	294.85±17.99	296.53±11.8	0.04
	24	**0.02**	**0.03**	**0.03**	**290.08±37.935**	**204.38±10.46**	**-1.13**	0.79	251.73±15.62	245.4±16.65	-0.17

	48	0.06	**0.01**	0.59	257.96±17.2	275.34±29.06	0.41	0.78	232.65±18.98	240.12±15.53	0.16
sucrose GC-MS	0	**0.03**									
	16	0.63	0.26	0.09	11112.03±1023.09	9153.15±994.31	-3.28	0.89	11415.36±561.3	11230.98±1303.31	-0.13
	24	0.15	0.68	0.06	8161.66±460.39	6272.26±643.66	-2.05	0.55	11000.52±893.92	12350.32±1964.13	0.62
	48	0.55	0.23	0.25	8241.46±385.44	7211.88±818.24	-1.09	0.31	12354±1535.16	9557.38±1985.77	-0.74
sugar GC-MS	0	0.41									
	16	0.08	0.06	**0.01**	**1803.33±130.84**	**1097.9±44.17**	**-1.57**	0.09	1310.05±166.68	957.27±42.8	-0.86
	24	0.61	0.61	0.06	1108.05±80.325	1524.1±141.62	2.59	0.51	1616.16±155.19	1862.77±325.53	0.65
	48	0.91	0.03	0.13	1041.85±38.56	1343.75±194.35	3.2	0.23	1839.82±253.18	1403.11±191.82	-0.7
sugar GC-MS	0	**< 0.01**									
	16	0.01	**< 0.01**	**0.02**	**56.06±5.34**	**78.01±7.77**	**5.52**	0.12	110.65±18.25	151.96±14.7	0.92
	24	0.82	0.26	**0.03**	**124.99±29.215**	**60.98±2.95**	**-1.1**	0.78	110.66±16.7	117.04±14.61	0.16
	48	0.72	0.76	**< 0.01**	**91.79±14.16**	**166.12±12.51**	**2.14**	0.32	109.15±22.92	146.9±26	0.67
sugar GC-MS	0	0.06									
	16	**0.01**	**0.02**	0.2	232.15±44.32	203.01±21	-2.75	0.77	294.44±20.73	303.95±23.61	0.19
	24	0.94	0.27	**0.01**	**412.55±87.9**	**151.39±8.23**	**-1.49**	0.15	291.55±28.4	225.72±31.47	-0.95
	48	0.42	0.14	0.12	350.91±46	448.51±36.96	0.87	0.46	260.05±52.21	315.52±42.66	0.43
sugar GC-MS	0	**0.01**									
	16	**0.02**	**< 0.01**	0.53	128.17±14.1	121.33±9.71	-0.73	0.09	187.91±20.93	245.93±21.43	1.13

	24	**< 0.01**	**< 0.01**	**0.03**	**215.51±52.08**	**97.55±9.76**	**-1.13**	**0.01**	**140.15±10.89**	**185.47±9.18**	**1.7**
	48	**< 0.01**	**< 0.01**	**< 0.01**	**134.54±19.8**	**240.08±23.43**	**2.18**	0.34	156.59±21.6	195.8±31.4	0.74
sugar GC-MS	0	0.44									
	16	**0.03**	0.65	**0.01**	**1176.93±64.57**	**1573.43±89.74**	**2.3**	0.37	1507.15±104.29	1627.12±60.19	0.47
	24	0.69	0.07	0.12	1609.27±192.225	1320.38±56.81	-0.75	0.61	1352.45±137.49	1267.66±88.11	-0.25
	48	0.18	0.21	0.17	1464.65±104.79	1288.21±63.21	-0.69	0.64	1323.45±121.47	1243.15±100.43	-0.27
sugar GC-MS	0	0.81									
	16	0.1	0.85	**0.02**	**106.49±11.44**	**184.04±27.57**	**7.73**	0.25	139.06±17.4	176.12±25.76	0.87
	24	0.28	0.28	0.14	160.62±33.25	110.71±12.08	-0.75	0.55	104.04±12.73	114.16±10.38	0.32
	48	0.24	0.52	0.09	102.39±11.07	139.23±17.93	1.36	0.65	119.32±8.44	110.88±15.86	-0.41
sugar GC-MS	0	0.44									
	16	0.42	0.47	**0.01**	**248.26±21.03**	**170.62±20.22**	**-4.79**	0.91	189.75±69.39	199.7±31.78	0.06
	24	0.69	0.07	**< 0.01**	**391.37±84.42**	**96.6±11.99**	**-1.75**	0.37	305.64±93.14	194.82±71.41	-0.49
	48	**0.05**	0.65	**0.02**	**164.12±21.98**	**422.57±100.32**	**4.8**	0.46	190.55±83.51	283.03±75.75	0.45
sugar GC-MS	0	**0.02**									
	16	0.69	0.23	**< 0.01**	**513.73±239.04**	**157.07±26.64**	**-5.36**	0.38	458.31±133.64	297.92±103.3	-0.49
	24	0.17	**0.04**	**< 0.01**	**804.91±228.345**	**54±9.33**	**-1.64**	0.1	504.23±118.75	235.57±90.53	-0.92
	48	0.11	**0.01**	0.82	236.96±110.3	208.8±58.91	-0.1	0.08	197.05±35.13	108.63±22.3	-1.03

sugar (cellobiose) GC-MS	0	0.81									
	16	0.73	0.49	0.62	1180.01±183.36	1024.16±258.26	-0.45	0.28	1089.31±209.1	806.74±101.16	-0.55
	24	0.26	0.24	0.47	855.32±237.43	693.1±84.54	-0.34	0.61	1148.72±237.46	976.92±230.12	-0.3
	48	0.14	0.96	0.28	623.66±105.41	755.02±47.31	0.51	**0.02**	**1282.25±197.47**	**600.05±71.06**	**-1.41**
sugar (pyranose) GC-MS	0	0.46									
	16	0.06	0.16	0.14	770.16±172.51	1057.84±174.56	3.71	**0.01**	**945.35±75.4**	**1441.08±154.96**	**2.68**
	24	**< 0.01**	**< 0.01**	**0.02**	**1944.16±461.165**	**800.54±102.15**	**-1.24**	0.12	935.99±71.09	1068.51±33.33	0.76
	48	**0.01**	**< 0.01**	**< 0.01**	**790.44±130.18**	**1937.97±301.51**	**3.6**	0.86	1294.93±264.46	1370.79±286.8	0.12
sugar (pyranose) GC-MS	0	0.39									
	16	0.67	0.86	**0.01**	**384.4±37**	**126.61±28.83**	**-1.68**	**0.04**	**344.79±64.13**	**137.34±51.52**	**-1.32**
	24	0.28	0.36	0.93	427.13±227.27	405.54±118.92	-0.05	0.35	176.35±62.72	269.35±70.44	0.61
	48	0.91	0.36	0.65	178.46±41.09	226.59±102.2	0.48	**0.05**	**447.43±127.43**	**125.36±34.54**	**-1.03**
threose GC-MS	0	0.62									
	16	0.51	0.1	**< 0.01**	**82±14.08**	**38.04±2.13**	**-4.17**	0.11	92.08±14.04	59.18±10.93	-0.96
	24	0.08	**< 0.01**	**0.01**	**63.34±4.035**	**39.04±4.51**	**-3.01**	0.2	64.48±9.04	91.74±17.42	1.23
	48	0.59	0.11	0.59	47.03±2.22	42.62±8.38	-0.81	0.14	83.14±17.13	51.08±6.14	-0.76
unknown disaccharide GC-MS	0	0.58									
	16	**0.03**	0.61	**< 0.01**	**385.8±12.31**	**177.3±41.4**	**-4.24**	**0.04**	**278.25±39.14**	**146.82±35.6**	**-1.37**
	24	0.55	0.09	0.62	232.58±51.345	201.41±35.39	-0.3	0.8	245.02±42.7	261.31±45.12	0.16

	48	0.31	0.14	0.43	173.13±42.64	134.31±21.12	-0.37	0.76	205.7±33.14	189.34±36.78	-0.2
unknown, putative sugar GC-MS	0	0.55									
	16	0.33	**< 0.01**	0.86	1825.86±186.55	1804.76±17.55	-0.08	0.09	1992.88±125.51	2299.27±95.24	1
	24	0.35	**0.02**	**0.04**	**2206.72±210.095**	**1698.72±90.65**	**-1.21**	0.97	1866.32±98.3	1860.93±70.47	-0.02
	48	0.27	**0.02**	**< 0.01**	**1620.04±155.58**	**2531.18±94.73**	**2.39**	0.46	2050.93±152.91	2208.84±119.42	0.42
xylulose GC-MS	0	**0.03**									
	16	0.18	0.1	0.28	572.3±401.17	989.37±348.69	1.73	0.28	1326.64±520.11	2182.19±521.3	0.67
	24	**0.02**	0.45	**0.01**	**3093.94±1071.845**	**106.57±38.93**	**-1.39**	0.29	1854.01±740.71	844.97±514.94	-0.56
	48	**0.04**	0.06	**0.01**	**1819.69±458.18**	**4047.58±551.96**	**1.99**	0.3	1115.32±599.26	2070.35±576.89	0.65

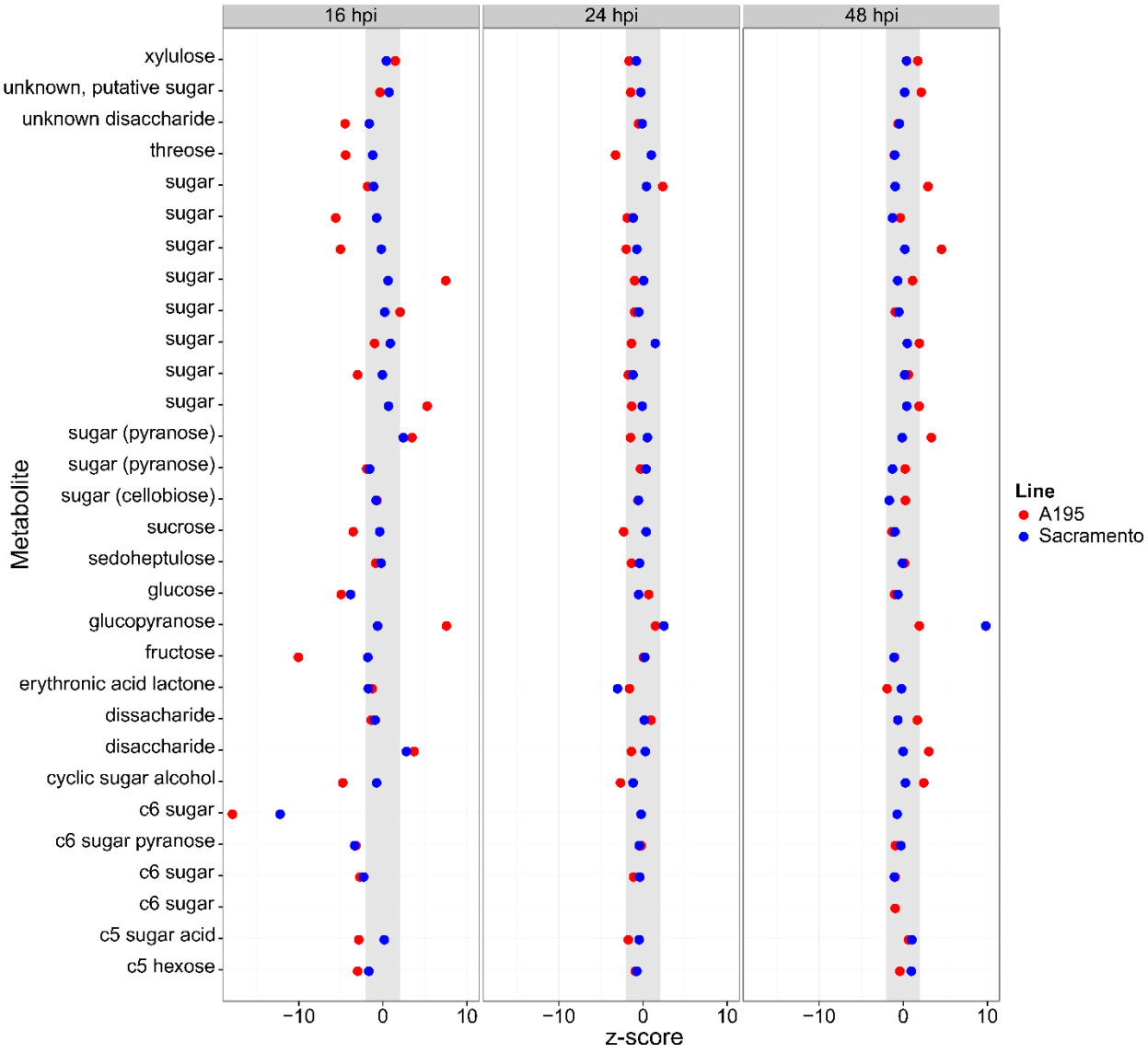

Figure 15: Z scores that illustrate the relative changes in sugar abundance at 16, 24, and 48 hpi in A195 and Sacramento when challenged with virulent inoculum relative to mock inoculum. Grey regions indicate areas of insignificant data points.

Resistance to *S. sclerotiorum* is associated with variation in fatty acids and lipids at early time points

Fatty acid relative abundance responded differently in A195 and Sacramento in response to virulent inoculum (Table 11). Fatty acid abundance increased in A195 at 16 hpi with virulent inoculum compared to mock inoculum with the exception that no change occurred in eicosanoic acid and α-Linolenic acid. Fatty acids did not change in Sacramento at 16 hpi with virulent inoculum compared to mock with the exception of a decrease in eicosanoic. There was a shift in A195 from the production of stearic acid and palmitic acid at 16 and 24 hpi to glyceropalmitic acid and α-Linolenic acid at 48 hpi in response to virulent inoculum compared to mock (Fig. 16). Fatty acids did not change in Sacramento at 24 or 48 hpi with virulent inoculum compared to mock. A195 and Sacramento showed different responses in fatty acids in the presence of virulent inoculum. The change in α-Linolenic acid and other fatty acids may contribute to resistance mechanism in A195.

Table 11: Changes in fatty acid relative abundance at 0, 16, 24, and 48 hpi with virulent and mock inoculum in A195 and Sacramento represented as Studentized t-test, means ± se ABU, and z-scores.

		A195 vs Sacramento		A195				Sacramento			
Metabolite	**hpi**	**mock†**	**inoc†**	**inoc vs. mock†**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score ††**	**inoc vs. mock†**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score ††**
dodecanoic acid GC-MS	0	0.07									
	16	0.06	**0.02**	**< 0.01**	**8.84±0.72**	**13.73±0.77**	**5.35**	0.94	10.31±0.58	10.24±0.8	-0.05
	24	0.32	0.06	0.81	11.87±1.18	12.24±0.94	0.16	0.84	10.06±0.21	9.85±0.98	-0.4
	48	0.35	0.32	0.61	13.67±0.98	14.92±2.35	0.52	0.42	9.99±0.37	11.43±1.69	1.6
eicosanoic acid GC-MS	0	0.19									
	16	**< 0.01**	0.82	**0.01**	**9.87±1.18**	**12.34±0.38**	**1.92**	**< 0.01**	**15.69±0.38**	**12.54±0.72**	**-3.37**
	24	0.23	**< 0.01**	0.19	11.45±0.6	12.56±0.48	0.93	0.17	16.43±0.65	15.02±0.71	-0.89
	48	0.36	0.13	0.98	12.9±0.87	12.86±1.12	-0.02	0.94	14.28±1.38	14.44±1.3	0.05
glyceropalmitic acid GC-MS	0	**0.03**									
	16	0.06	0.22	**0.01**	**13.48±1.09**	**17±0.82**	**2.49**	0.96	14.95±0.4	15.01±1.19	0.06
	24	0.43	0.27	0.11	18.95±2.67	14.9±0.62	-0.76	0.97	14.32±0.76	14.36±0.66	0.02
	48	**0.01**	0.1	**< 0.01**	**16.94±1.32**	**28.43±2.21**	**3.55**	**0.03**	**14.2±1.55**	**19.05±0.93**	**1.28**
linolenic acid GC-MS	0	**< 0.01**									
	16	**< 0.01**	**0.02**	**0.05**	**328.25±12.12**	**382.02±20.27**	**1.81**	0.32	832.24±67.32	700.34±110.44	-0.8
	24	0.14	0.19	0.75	428.9±46.875	446.95±32.16	0.19	0.67	937.66±91.41	879.6±97.41	-0.26
	48	0.08	0.06	**< 0.01**	**367.62±28.23**	**576.26±30.49**	**3.02**	0.47	850.49±104.25	745.47±78.23	-0.41
palmitic acid GC-MS	0	0.15									
	16	**< 0.01**	0.3	**< 0.01**	**970.47±85.42**	**1513.69±68.91**	**7.6**	0.6	1237.9±63.02	1322.99±155.18	0.55
	24	0.56	0.08	**0.01**	**1189.72±24.89**	**1489.27±74.43**	**6.02**	0.52	1174.09±41.8	1242.17±93.89	0.66
	48	**0.05**	0.51	0.72	1328.55±101.46	1288.68±43.05	-0.16	0.59	1190.72±46.94	1247.09±86.57	0.49
stearic acid GC-MS	0	0.15									
	16	**< 0.01**	0.11	**< 0.01**	**400.73±68.64**	**754.32±32.62**	**6.46**	0.84	563.28±36.42	581.76±89.31	0.21
	24	0.14	0.7	**< 0.01**	**530.8±12.655**	**716.17±36.17**	**7.33**	0.64	535.04±18.11	555.11±36.93	0.45
	48	0.19	**< 0.01**	0.23	632.68±62.11	543.8±32.87	-0.58	0.66	513.42±31.59	540.88±49.39	0.35

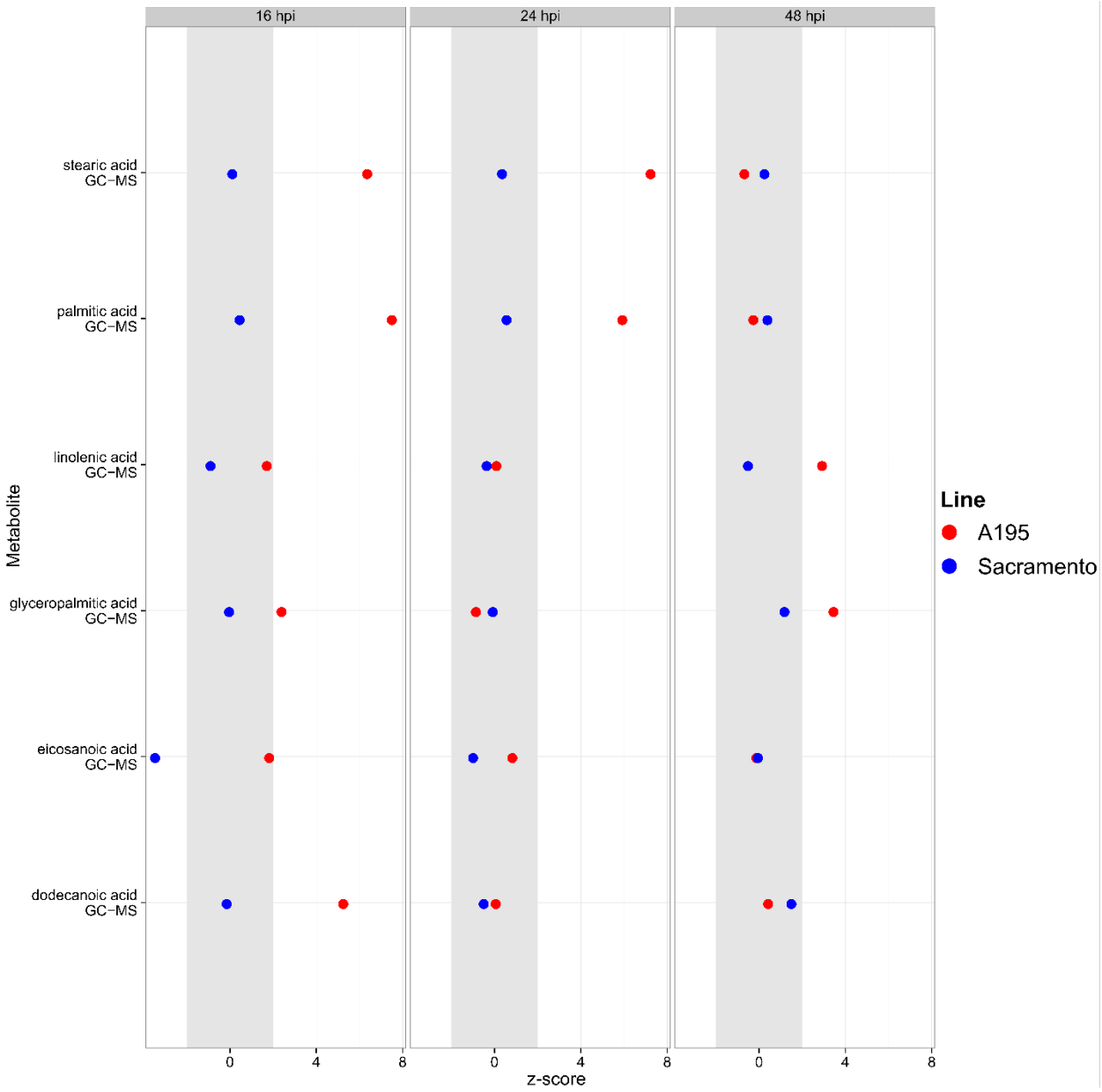

Figure 16: Z scores that illustrate the relative changes in fatty acid abundance at 16, 24, and 48 hpi in A195 and Sacramento when challenged with virulent inoculum relative to mock inoculum. Grey regions indicate areas of insignificant data points.

Several unknown lipid signals were detected. The relative abundance of these lipids responded differently in A195 and Sacramento in response to virulent inoculum (Table 12). Lipid abundance increased in A195 at 16 hpi with virulent inoculum compared to mock. There was no change in lipid abundance at 24 or 48 hpi after virulent inoculation in A195 compared to mock. Sacramento showed no change in lipids with the exception of one unknown lipid at 24 hpi in response to virulent inoculum compared to mock (Fig. 17). Lipids in A195 and Sacramento differed in reaction to virulent inoculum. An initial change in lipid abundance may be involved in a resistance mechanism to A195.

Table 12: Changes in lipid relative abundance at 0, 16, 24, and 48 hpi with virulent and mock inoculum in A195 and Sacramento represented as Studentized t-test, means ± se ABU, and z-scores.

		A195 vs Sacramento		A195				Sacramento			
Metabolite	**hpi**	**mock †**	**inoc†**	**inoc vs. mock†**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score ††**	**inoc vs. mock†**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score ††**
isopentenyl diphosphate LC-MS	0	0.59									
	16	0.27	**< 0.01**	**0.01**	**6.39±0.6**	**9.94±0.77**	**2.43**	0.71	4.89±1.13	4.39±0.34	-0.18
	24	0.02	0.76	0.22	7.36±1.17	11.55±1.81	1.79	**0.01**	**4.26±0.5**	**7.88±0.99**	**2.94**
	48	0.31	0.26	0.82	9.48±0.55	9.81±1.48	0.25	0.25	9.6±1.27	7.83±0.36	-0.57
lipid LC-MS	0	0.09									
	16	**0.05**	0.11	**0.01**	**412.98±13.1**	**317.77±38.27**	**-2.97**	0.14	304.23±48.02	459.93±88.3	1.32
	24	0.51	0.21	0.83	319.75±41.535	291.49±48.95	-0.34	**0.04**	**286.99±17.84**	**380.89±35.63**	**2.15**
	48	0.12	0.1	0.83	216.75±20.8	229.94±69.51	0.26	0.6	329.27±36.22	296.36±46.65	-0.37
multiple lipids LC-MS	0	**0.01**									
	16	**< 0.01**	0.16	**0.01**	**2.66±0.24**	**4.22±0.27**	**2.67**	0.14	6.68±0.79	5.03±0.58	-0.85
	24	0.3	0.63	0.2	2.85±0.255	3.31±0.3	0.91	0.9	6.81±0.6	6.68±0.74	-0.08
	48	0.4	0.71	0.58	3.13±0.28	3.35±0.29	0.31	0.91	6.2±0.71	6.06±0.83	-0.08
multiple lipids LC-MS	0	0.95									
	16	0.22	0.95	**< 0.01**	**48.31±3.27**	**89.92±9.12**	**5.19**	0.18	61.52±9.5	88.42±16.82	1.16
	24	**0.04**	0.21	0.12	57.57±8.725	71.94±5.76	0.82	0.98	54.27±9.83	54.77±13.31	0.02
	48	0.07	0.71	0.66	49.67±8.16	45.07±6.74	-0.23	0.65	54.79±11.46	48.11±6.7	-0.24
multiple lipids LC-MS	0	0.19									
	16	0.58	0.19	**0.01**	**10.44±0.64**	**15.44±1.13**	**3.21**	0.19	9.6±1.29	12.35±1.41	0.87
	24	0.26	0.09	0.96	16.09±5.63	10.79±0.69	-0.47	0.52	8.81±0.64	10.01±1.69	0.77
	48	0.89	0.61	0.08	9.73±1.07	7.32±0.71	-0.91	0.83	8.8±1.02	8.51±0.65	-0.11
multiple lipids LC-MS	0	0.44									
	16	0.2	0.85	**< 0.01**	**15.47±0.62**	**22±0.93**	**4.26**	0.3	18.3±1.96	21.56±2.25	0.68
	24	0.3	0.07	0.1	15.9±2.31	20.27±1.23	0.95	0.46	17.29±1.45	19.11±1.87	0.51
	48	**< 0.01**	0.18	**0.05**	**16.28±1.21**	**12.99±1.06**	**-1.11**	0.95	16.53±1.84	16.39±0.93	-0.03
multiple lipids LC-MS	0	**< 0.01**									
	16	**< 0.01**	0.07	**< 0.01**	**20.78±0.56**	**31.14±2.4**	**7.62**	0.07	30.19±2.44	36.44±1.5	1.05
	24	**0.03**	**0.03**	0.06	20.11±3.13	24.78±0.78	0.75	0.18	29.76±1.65	26.3±1.77	-0.85

	48	0.12	0.21	0.99	18.31±1.89	18.27±1.66	-0.01	0.67	26.86±3.18	24.91±2.6	-0.25
multiple lipids LC-MS	0	0.1									
	16	0.76	0.4	**0.01**	**24.51±1.74**	**46.79±6.47**	**5.23**	0.3	25.97±4.35	35.81±8.35	0.92
	24	0.89	0.06	0.2	32.68±6.83	31.9±2.59	-0.06	0.98	24.72±3.36	24.55±5.93	-0.02
	48	0.19	0.6	**0.01**	**25.84±2.11**	**17.62±1.71**	**-1.59**	0.86	21.02±3.23	20.13±3.46	-0.11
multiple lipids LC-MS	0	0.42									
	16	0.51	0.88	**< 0.01**	**8.75±0.47**	**12±0.67**	**2.82**	0.08	9.32±0.7	12.32±1.45	1.75
	24	0.08	0.56	0.58	11.22±2.045	10.08±0.25	-0.28	0.25	8.81±0.69	10.6±1.29	1.06
	48	0.21	**< 0.01**	0.03	10.23±0.69	7.93±0.69	-1.35	0.93	8.36±0.73	8.45±0.62	0.05
multiple lipids LC-MS	0	0.1									
	16	**< 0.01**	0.56	**0.01**	**6.77±0.37**	**9.45±0.67**	**2.99**	0.25	11.7±1.29	9.76±0.7	-0.61
	24	0.37	0.18	**0.04**	**6.93±1.27**	**8.73±0.51**	**0.71**	0.47	12.39±1.37	14.32±2.17	0.58
	48	0.68	0.98	0.15	7.7±0.31	7±0.32	-0.94	0.69	11.1±1.6	12.1±1.62	0.25
multiple lipids LC-MS	0	0.4									
	16	0.12	0.58	**0.02**	**499.52±45.23**	**947.52±157.23**	**4.81**	0.32	800.49±173.26	1134.39±275.43	0.79
	24	0.31	0.16	0.46	589.66±168.17	748.32±135.15	0.47	0.97	658.63±154.8	649.66±175.79	-0.02
	48	0.96	0.93	0.35	414.7±121.39	279.6±69.38	-0.45	0.35	646.32±154.95	457.16±91.77	-0.5

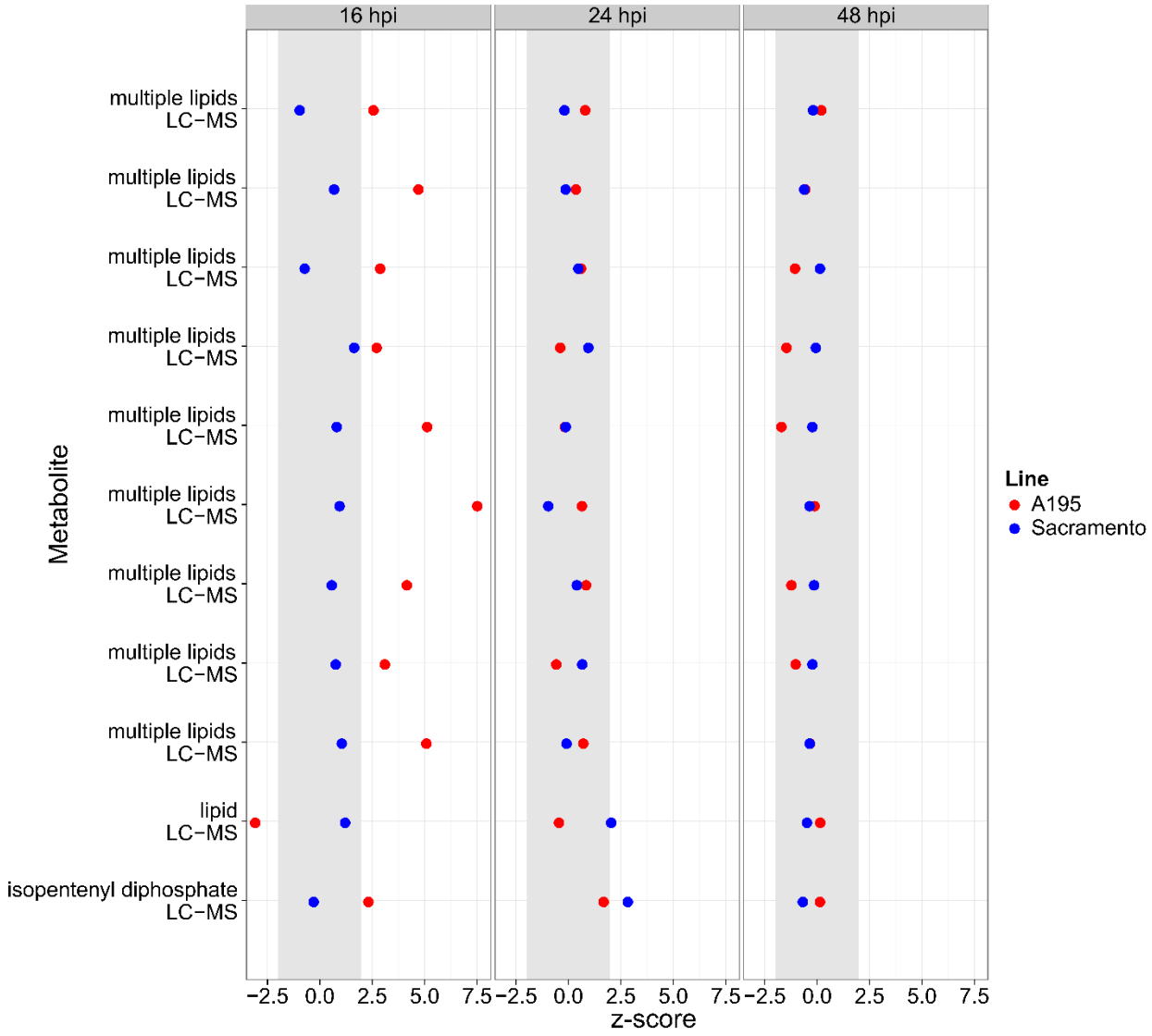

Figure 17: Z scores that illustrate the relative changes in lipid abundance at 16, 24, and 48 hpi in A195 and Sacramento when challenged with virulent inoculum relative to mock inoculum. Grey regions indicate areas of insignificant data points.

***S. sclerotiorum* was associated with phytoalexins, but not flavonoids**

Flavonoids react similarly in A195 and Sacramento in response to virulent inoculum compared to mock inoculum (Table 13). There was an increase in relative abundance in flavonoids apigenin and luteolin in both A195 and Sacramento at 16 hpi with virulent inoculum compared to mock inoculum (Fig. 18). Luteolin continued to increase in abundance at 24 and 48 hpi with virulent inoculum compared to mock in both A195 and Sacramento. Production of apigenin is different between A195 and Sacramento in response to virulent inoculum at 24 hpi ($p < 0.001$) and 48 hpi ($p = 0.01$). Relative abundance of flavonoids in A195 and Sacramento respond similarly to virulent inoculum compared to mock inoculum.

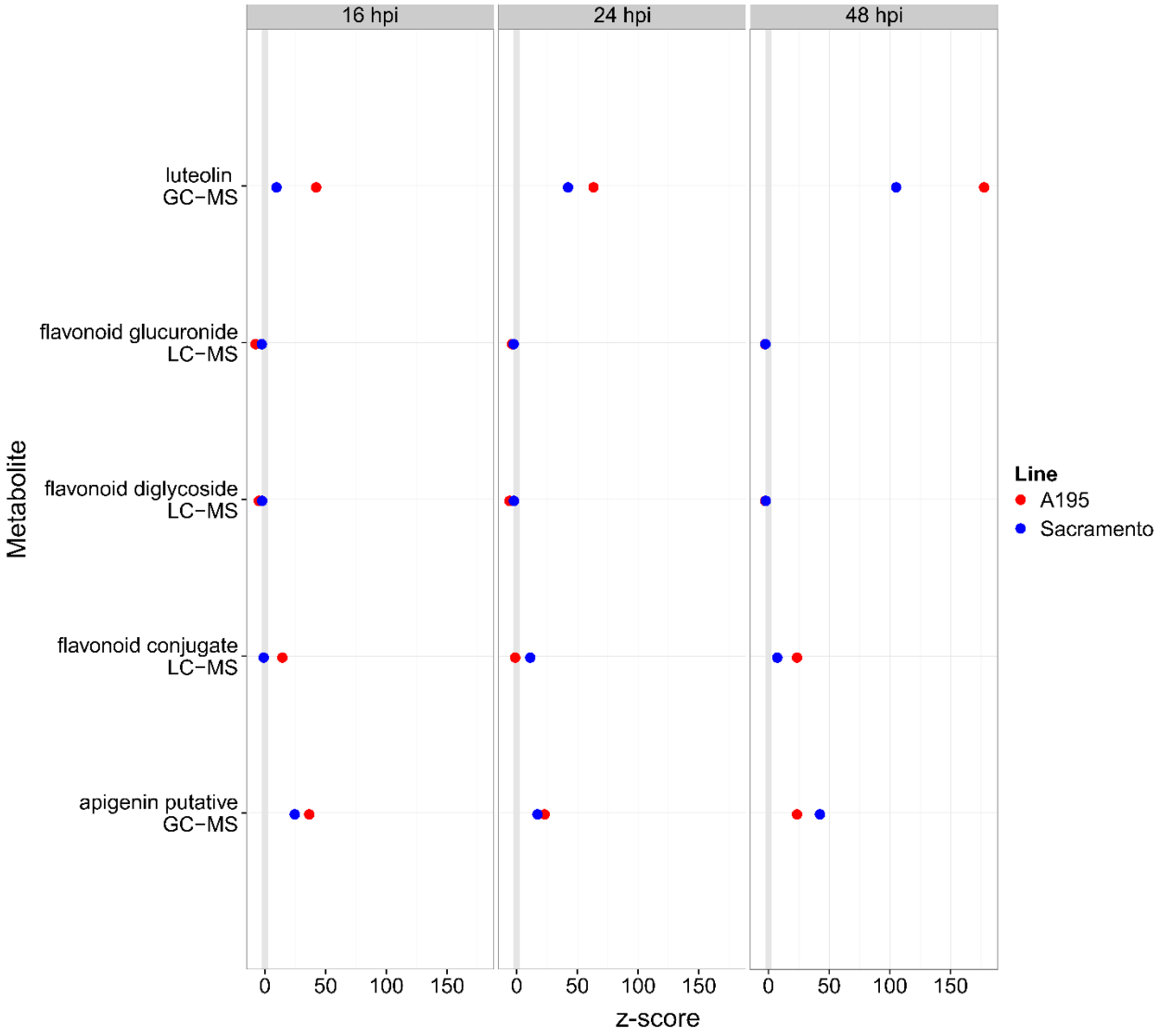

Figure 18: Z scores that illustrate the relative changes in flavonoid abundance at 16, 24, and 48 hpi in A195 and Sacramento when challenged with virulent inoculum relative to mock inoculum. Gray regions indicate areas of insignificant data points.

Table 13: Changes in flavonoid relative abundance at 0, 16, 24, and 48 hpi with virulent and mock inoculum in A195 and Sacramento represented as Studentized t-test, means ± se ABU, and z-scores.

		A195 vs Sacramento		A195				Sacramento			
Metabolite	**hpi**	**mock†**	**inoc†**	**inoc vs. mock†**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score††**	**inoc vs. mock†**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score††**
apigenin, putative GC-MS	0	0.08									
	16	0.25	0.28	**< 0.01**	**1.29±0.4**	**12.55±2.38**	**38.4**	**< 0.01**	**1.49±0.11**	**8.64±2.13**	**26.45**
	24	**0.01**	**< 0.01**	0.11	1.98±0.37	20.33±8.25	24.88	**0.01**	**1.69±0.24**	**13.08±3.79**	**19.01**
	48	**0.01**	**0.01**	0.07	2.38±0.24	17.62±8.14	25.36	0.08	1.79±0.21	24.16±11.51	44.26
flavonoid conjugate LC-MS	0	0.03									
	16	0.89	**< 0.01**	**< 0.01**	**6.61±0.49**	**26.32±2.99**	**16.27**	0.22	6.86±1.58	10.11±1.96	0.84
	24	0.77	0.1	0.54	19.37±8.02	29.45±9.86	0.63	0.07	7.59±0.93	37.18±14.81	13.03
	48	0.15	0.59	**0.04**	**14.84±1.89**	**132.16±59.93**	**25.39**	**0.02**	**9.58±2.01**	**54.61±15.86**	**9.13**
flavonoid diglycoside LC-MS	0	0.01									
	16	0.79	0.12	**0.02**	**244.11±12.89**	**144.85±22.18**	**-3.14**	0.41	254.34±35	217.99±17.9	-0.42
	24	0.67	**< 0.01**	**0.01**	**441.17±29.735**	**201.14±51.16**	**-4.04**	0.58	260.47±14.19	246.37±20.02	-0.41
	48	0.86	0.12	0.09	436.79±71.24	293.37±28.69	-0.82	0.46	309.69±44.58	270.84±12.38	-0.36
flavonoid glucuronide LC-MS	0	**< 0.01**									
	16	**< 0.01**	0.33	**< 0.01**	**11308.95±504.85**	**4043.9±840.47**	**-5.87**	0.21	5224.39±924.95	3694.58±520.48	-0.68
	24	0.16	0.98	**0.05**	**12189.43±1380.99**	**6780.99±1634.08**	**-1.96**	0.22	5455.32±826.39	4146.02±581.45	-0.65
	48	0.82	**0.05**	0.06	11115.07±1406.19	8014.53±560.52	-0.9	0.25	5768.03±515.77	4952.53±334.22	-0.65
luteolin GC-MS	0	0.28									
	16	0.44	0.15	**0.02**	**0.59±0.12**	**8.25±2.65**	**44.07**	**< 0.01**	**0.69±0.1**	**3.42±0.8**	**11.4**
	24	0.76	0.09	**0.05**	**0.73±0.08**	**10.95±3.6**	**65.14**	**0.05**	**0.66±0.1**	**11.09±4.66**	**44.17**
	48	0.58	0.1	**0.01**	**1.11±0.16**	**70.5±24.13**	**179.56**	0.1	0.83±0.14	36.82±19.77	107.09

Relative abundance in secondary metabolites responded differently in A195 and Sacramento to virulent inoculum (Table 14). In regards to secondary metabolism, A195 had an overall greater response in abundance to virulent inoculum compared to Sacramento. Kievitone and phaseollin/abyssinone I increased in both A195 and Sacramento (Fig. 19). Kievitone was increased in A195 compared to Sacramento at 24 hpi ($p < 0.001$) and 48 hpi ($p = 0.01$) in response to virulent inoculum. Soyasaponin increased in A195 at 16, 24 and 48 hpi in response to virulent inoculum compared to mock. Soyasaponin decreased in Sacramento 16 hpi in response to virulent inoculum compared to mock. Soyasaponin was more abundant in A195 compared to Sacramento at 24 hpi ($p = 0.01$) and 48 hpi ($p = 0.05$) in response to virulent inoculum. Coumestrol slightly increased in A195 in response to virulent inoculum compared to mock (Fig. 19). Coumestrol differed in relative abundance between A195 and Sacramento at 24 hpi ($p < 0.001$) in response to virulent inoculum. A195 and Sacramento respond differently to virulent inoculum in regards to secondary metabolism. A195 secondary metabolites were in a greater abundance compared to Sacramento in response to virulent inoculation, possibly providing a resistance mechanism.

Table 14: Changes in secondary metabolites relative abundance at 0, 16, 24, and 48 hpi with virulent and mock inoculum in A195 and Sacramento represented as Studentized t-test, means ± se ABU, and z-scores.

		A195 vs Sacramento		A195				Sacramento			
Metabolite	**hpi**	**mock †**	**inoc†**	**inoc vs. mock †**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score† †**	**inoc vs. mock†**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score †† **
coumestrol LC-MS	0	**< 0.01**									
	16	0.06	0.17	0.21	5.89±1.39	19.42±8.24	3.98	0.49	2.94±0.25	3.35±0.55	0.66
	24	0.42	**< 0.01**	0.13	6.11±2.135	47.04±15.95	9.59	0.23	3.32±0.61	8.12±3.74	3.19
	48	0.01	0.07	**< 0.01**	**6.46±0.52**	**155.36±41.51**	**117.3**	0.07	4.22±1.17	14.48±4.82	3.59
kievitone LC-MS	0	0.97									
	16	0.21	0.22	0.25	8.19±2.6	34.98±17.44	4.21	0.57	4.63±0.6	4.21±0.33	-0.29
	24	**0.04**	**< 0.01**	0.09	4.73±0.53	88.85±28.86	79.15	0.08	4.64±0.86	46.46±21.78	19.87
	48	**0.01**	**0.01**	**0.01**	**9.69±2.06**	**610.71±229.29**	**118.97**	0.2	7.04±2.36	198.76±141.09	33.11
nerolidol tri-glycoside LC-MS	0	**0.01**									
	16	**< 0.01**	0.44	0.47	779.68±85.13	896.42±123.22	0.78	**0.03**	**1930.95±94.19**	**1165.17±305.97**	**-3.32**
	24	0.22	0.16	0.14	814.56±133.14	495.44±74.17	-1.2	0.66	1980.66±187.79	1820.8±304.09	-0.35
	48	0.57	0.55	0.85	642.36±63.45	665.15±105.79	0.15	0.9	1474.59±260.7	1523.47±257.34	0.08
phaseolin/ abyssinone i LC-MS	0	0.79									
	16	0.14	0.14	0.14	12.15±3.13	68.19±27.44	7.31	0.46	7.06±0.69	8.4±1.74	0.79
	24	0.14	0.23	0.08	11.44±3.82	137.48±42.39	16.49	0.07	7.8±1.7	20.8±6.16	3.11
	48	0.35	0.82	**< 0.01**	**17.46±3.2**	**617.75±108.76**	**76.59**	**0.04**	**11.39±4.16**	**105.43±39.96**	**9.22**
phaseollidin/ phaseollin-isoflavan LC-MS	0	0.15									
	16	0.11	0.34	0.06	49.22±8.83	78.81±25.15	1.37	0.65	85.14±18.74	73.2±16.11	-0.26
	24	0.28	0.18	0.33	69.51±13.025	118.6±24.26	1.88	0.25	103.04±28.96	66.15±9.03	-0.52
	48	0.12	**0.02**	0.44	84.55±19.6	103.3±12.71	0.39	0.16	56.1±9.5	80.86±12.15	1.06
quinic-acid like compound LC-MS	0	0.22									
	16	0.21	0.13	**0.05**	**699.57±30.64**	**978.86±122.58**	**4.17**	0.24	1003.56±225.28	1402.42±213.6	0.72
	24	**< 0.01**	0.16	0.15	930.1±146.46	603.99±100.79	-1.11	0.45	731.6±113.92	879.09±149.58	0.53
	48	**0.01**	**< 0.01**	0.18	744.95±163.49	1083.8±181.72	0.85	0.18	754.18±80.81	1110.3±229.16	1.8
soyasaponin i LC-MS	0	0.29									
	16	0.06	**0.01**	**0.01**	**28.84±1.92**	**59.72±9.82**	**6.57**	**0.04**	**58.51±14.06**	**20.68±3.1**	**-1.1**
	24	**0.03**	**0.05**	**0.05**	**84.35±22.28**	**28.46±5.35**	**-1.25**	**< 0.01**	**70.08±4.65**	**41.64±5.22**	**-2.5**

	48	0.63	0.47	0.09	83.11±6.54	125.44±24.89	2.64	0.14	39.42±12.39	65.98±9.35	0.88
unknown LC-MS	0	0.32									
	16	0.28	**< 0.01**	**< 0.01**	**39.18±0.75**	**122.35±16.09**	**45.09**	0.1	33.6±4.85	46.17±4.56	1.06
	24	**0.05**	0.07	0.54	87.65±25.49	107.38±12.25	0.39	**< 0.01**	**40.1±8.21**	**98.45±11**	**2.9**
	48	0.78	**0.01**	**0.05**	**60.67±10.13**	**157.81±24.14**	**3.92**	**< 0.01**	**54.34±10.37**	**239.11±27.38**	**7.27**

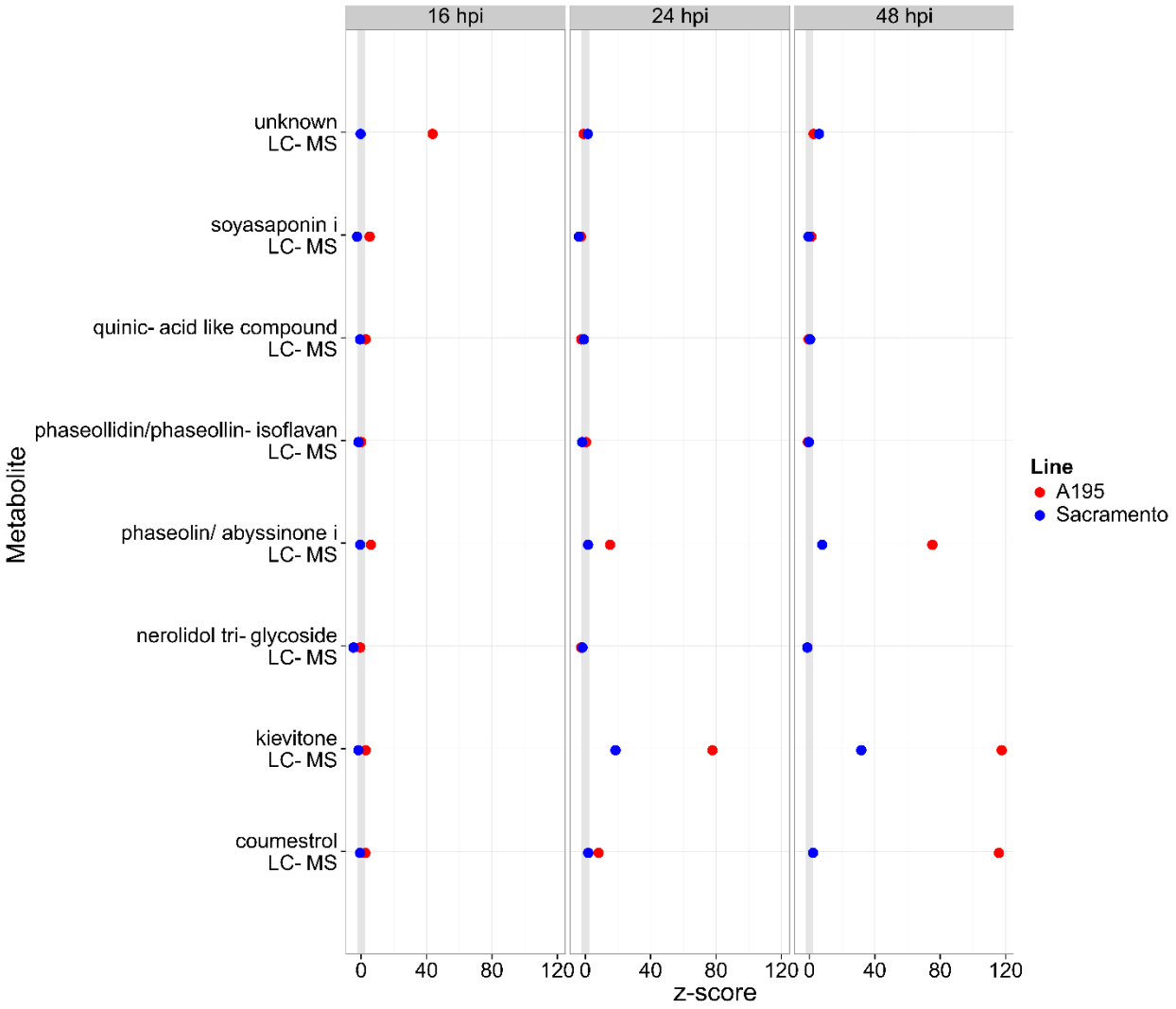

Figure 19: Z scores that illustrate the relative changes in secondary metabolite abundance at 16, 24, and 48 hpi in A195 and Sacramento when challenged with virulent inoculum relative to mock inoculum. Grey regions indicate areas of insignificant data points.

Phenylpropanoid pathway (PAL pathway)

Metabolites that increased in resistant barley (*Hordeum vulgare* L.) in response to *Fusarium* head blight [*Gibberella zeae*, (Schwein.) Petch] demonstrated that phenylpropanoid, fatty acids, flavonoids, and terpenoids have antifungal activity [34]. The first enzyme of the PAL pathway is phenylalanine ammonia-lyase, which converts phenylalanine into trans-cinnamic acid. This pathway is rapidly induced after pathogen infection and gives rise to important defense compounds such as phytoalexins, flavonoids, lignin and salicylic acid [62]. In bean cultivars infected with *S. sclerotiorum*, slower lesion development was associated with increased PAL activity [16]. Miklas et al. found that PAL activity increased in resistant compared to susceptible cultivars in common beans inoculated with *S. sclerotiorum* [49]. Compounds in the phenylpropanoid pathway are important for resistance to fungal pathogens.

Phytoalexins are antimicrobial compounds that are only induced upon pathogen invasion [34]. Grafton and McClean suggested that phytoalexins were likely to contribute to partial resistance to *S. sclerotiorum* [49]. Phytoalexins were more abundant in A195 than Sacramento at 16 hpi and continued to increase through later time points. Kievitone, phaseolin/abyssinone I, and coumestrol did not increase in abundance compared to the mock inoculum at 16 hpi in Sacramento in response to virulent inoculum, but increased at 24 and 48 hpi. Plant-pathogen interaction is determined not only on the extent of the response but also the speed of the response [63]. Phaseolin/abyssinone I is considered the first defense while plants biosynthesize additional phytoalexins [64]. Durango et al. found that Colombian bean cultivars resistant to the anthracnose fungus produced more phaseolin and coumestrol than

susceptible cultivars [64]. The production of phytoalexins in response to virulent inoculum in A195 and Sacramento is a possible resistant mechanism that may be exploited to improve disease resistance.

Flavonoids are polyphenols that originate in the PAL pathway. Phenolics, like flavonoids, create a toxic environment to fungi in plant tissue [63]. Chipps et al' [26] demonstrated that flavonoids increase in response to exposure to *S. sclerotiorum* and may contribute to resistance [10]. Flavonoids such as luteolin and apigenin were increased in response in both A195 and Sacramento, but with a higher increase in A195 (Fig. 20). The production of flavonoids is also a potential resistance mechanism that conditions a fungal toxic environment in A195 faster than in Sacramento.

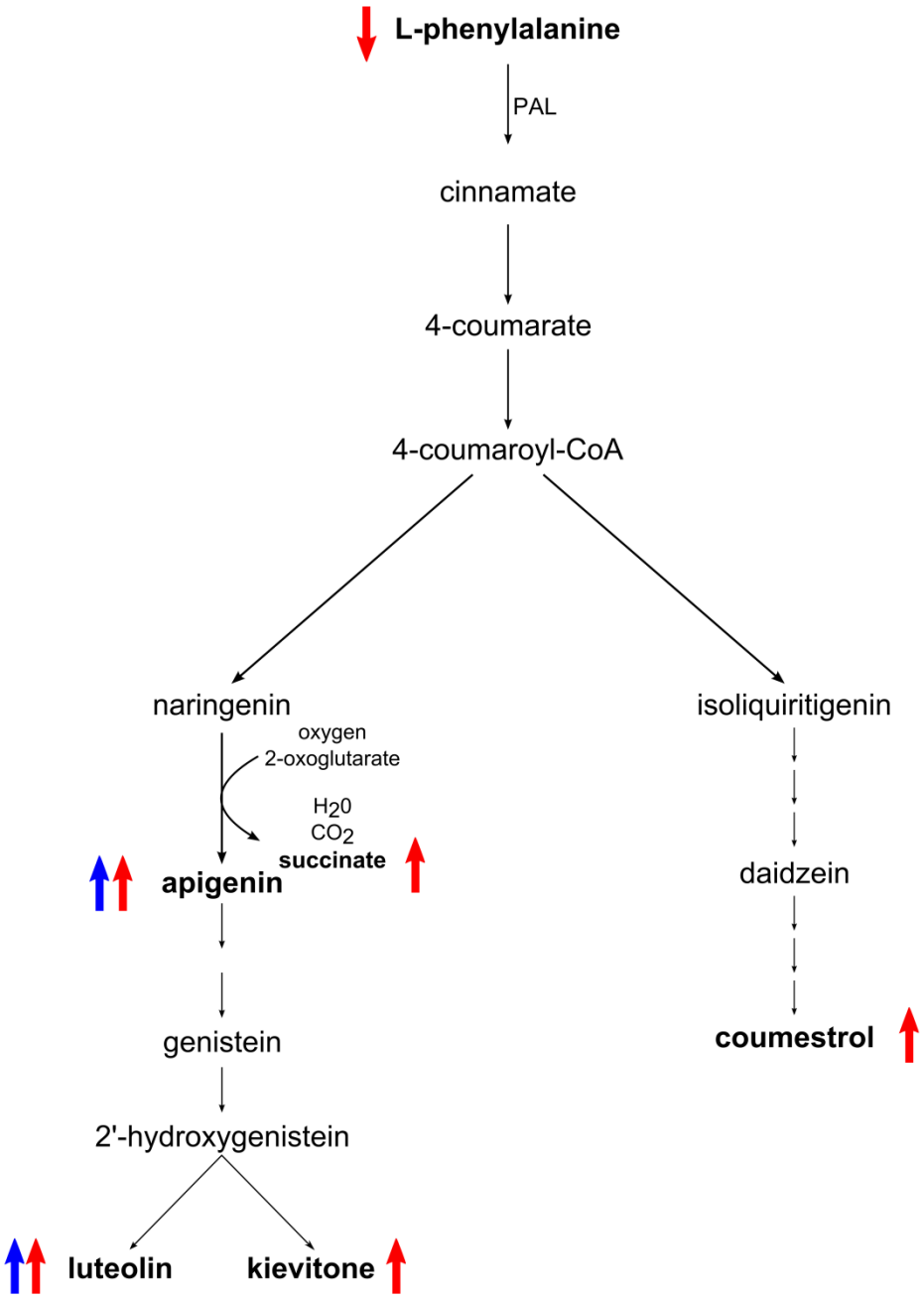

Figure 20: Phenylpropanoid pathway generating secondary metabolites in response to white mold [47]**.** Red arrows were metabolites increased or decreased in A195 post virulent inoculationand blue arrows were metabolites increased or decreased in Sacramento post virulent inoculation.

Relative abundance of unknown metabolites (no confident annotation) reacted differently in A195 and Sacramento in response to virulent inoculum (Table 15). Unknown metabolite abundance both increased and decreased in A195 at 16 hpi, with a diminished response at 24 and 48 hpi to virulent inoculum compared to mock (Fig. 21). In general there was no change in unknown metabolite abundance in Sacramento in response to virulent inoculation compared to mock. Unknown metabolite abundance changed more in A195 compared to Sacramento. The change in abundance of unknown metabolites in A195 happened at 16 hpi and not at later time points. These unknown metabolites may be involved in an initial resistance mechanism in A195.

Table 15: Changes in unknown metabolites relative abundance at 0, 16, 24, and 48 hpi with virulent and mock inoculum in A195 and Sacramento represented as Studentized t-test, means ± se ABU, and z-scores.

		A195 vs Sacramento		A195				Sacramento			
Metabolite	**hpi**	**mock †**	**inoc†**	**inoc vs. mock †**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score ††**	**inoc vs. mock †**	**mean mock ± se ABU‡**	**mean inoc ± se ABU‡**	**z-score ††**
ascorbalamic acid LC-MS	0	0.11									
	16	0.15	0.68	**< 0.01**	**152.96±7.09**	**66.69±4.92**	**-4.97**	0.08	126.99±15.07	79.31±19.1	-1.29
	24	**0.01**	**0.01**	0.71	76.27±14.655	69.89±10.92	-0.22	0.17	93.66±11.66	172.76±52.17	2.77
	48	**< 0.01**	**0.02**	0.7	50.17±6.12	46.12±9.87	-0.27	0.11	152.23±34.9	71.34±24.52	-0.95
m+h=291.106 LC-MS	0	0.3									
	16	**0.01**	**0.03**	**0.05**	**23.96±0.6**	**33.66±3.49**	**6.57**	0.54	40.12±4.5	43.57±2.47	0.31
	24	1	**< 0.01**	**0.05**	**97.7±36.095**	**19.65±1.35**	**-1.08**	0.95	38.88±5.57	38.45±4.38	-0.03
	48	**< 0.01**	0.65	**0.01**	**39.16±7.17**	**75.66±9.7**	**2.08**	0.57	45.36±8.6	52.88±8.7	0.36
sarmentoloside + 585.256 LC-MS	0	0.08									
	16	**0.01**	0.21	**< 0.01**	**32.4±5.26**	**87.52±10.72**	**4.28**	**0.05**	**321.67±82.24**	**115.69±18.36**	**-1.02**
	24	0.1	0.11	0.09	50.93±2.455	36.49±4.37	-2.94	**0.03**	**510.39±95.17**	**243.1±44.51**	**-1.15**
	48	**0.01**	0.26	0.09	46.5±4.87	35.79±3.77	-0.9	0.42	153.15±25.47	124.61±19.04	-0.46
similar to salvianolic acid LC-MS	0	0.96									
	16	0.17	0.89	**0.01**	**643.31±25.32**	**408.29±51.51**	**-3.79**	0.64	537.56±66.38	476.33±113.98	-0.38
	24	0.66	**< 0.01**	**0.02**	**1031.81±356.23**	**298.52±16.61**	**-1.03**	0.23	673.25±98.05	474.45±119.83	-0.83
	48	0.53	0.52	0.75	924.92±135.29	979.25±118.22	0.16	0.91	620.78±114.71	635.87±40.57	0.05
unknown LC-MS	0	0.08									
	16	**0.01**	**< 0.01**	**< 0.01**	8.68±0.33	14.2±0.85	6.81	0.18	16.32±2.14	20.23±1.34	0.74
	24	**0.03**	0.1	0.2	12.91±2.47	9.2±0.42	-0.75	0.94	15.29±2.58	14.99±3.08	-0.05
	48	0.57	0.19	0.13	11.21±1.08	13.3±0.67	0.79	0.58	13.9±2.26	15.48±1.16	0.29
unknown LC-MS	0	0.05									
	16	**< 0.01**	0.18	**< 0.01**	**2.95±0.25**	**8.74±1.25**	**9.43**	0.95	11.91±1.94	12.07±1.58	0.03
	24	0.54	0.93	0.18	4.45±1.985	12.59±3.11	2.05	0.42	13.71±1.67	16.66±3.09	0.72
	48	0.52	0.08	0.29	14.42±2.99	9.87±3.38	-0.62	0.98	20.66±3.12	20.86±6.37	0.03
unknown LC-MS	0	0.32									
	16	**0.02**	**0.03**	**0.01**	**20.94±1.95**	**72.04±15.64**	**10.69**	0.28	36.87±5.65	28.32±4.53	-0.62
	24	0.23	**< 0.01**	0.33	63.48±19.675	110.1±20.8	1.18	0.14	52.18±10.94	120.72±41.8	2.56

	48	**< 0.01**	0.36	0.35	56.38±9.11	45.34±6.56	-0.49	0.77	41.79±10.57	37.58±7.2	-0.16
unknown LC-MS	0	0.96									
	16	0.73	0.63	**< 0.01**	**254.57±10.28**	**173.84±14.27**	**-3.21**	0.66	242.42±32.57	213.61±56.68	-0.36
	24	0.6	0.82	**0.02**	**487.13±163.81**	**115.67±8.86**	**-1.13**	0.26	305.92±54.82	206.34±63.47	-0.74
	48	0.32	0.47	0.52	409.55±77.11	471.75±61.97	0.33	0.28	246.4±35.52	297.44±20.03	0.59
unknown LC-MS	0	0.09									
	16	**< 0.01**	0.45	**< 0.01**	**63.91±1.6**	**21.76±5.85**	**-10.74**	0.7	31.55±4.05	35.2±8.81	0.37
	24	**0.01**	0.38	0.11	34.52±13.32	25±1.58	-0.36	0.89	35.76±5.46	34.69±5.51	-0.08
	48	0.18	0.15	0.45	36.87±5.17	29.43±2.22	-0.59	0.6	43.23±9.3	50.51±8.48	0.32
unknown LC-MS	0	0.11									
	16	0.98	0.84	**0.02**	**30.49±1.35**	**51.7±6.07**	**6.4**	0.11	30.6±4.45	50.19±11.12	1.8
	24	0.1	0.67	0.12	32.46±5.44	37.34±2.38	0.45	0.75	26.36±2.39	29.18±8.15	0.48
	48	0.09	0.2	0.06	29.34±2.36	22.62±2.23	-1.16	0.88	28.74±4.95	27.59±4.7	-0.09
unknown LC-MS	0	0.83									
	16	0.08	0.83	**0.01**	**18.72±0.91**	**11.43±1.51**	**-3.26**	0.51	14.1±2.22	12.01±1.98	-0.38
	24	0.12	0.91	0.04	17.97±3.98	11.49±0.49	-0.81	0.55	11.98±0.51	13.09±1.74	0.89
	48	0.41	0.17	0.32	16.73±0.98	14.97±1.44	-0.73	0.69	13.81±2.81	12.5±0.8	-0.19
unknown LC-MS	0	**0.02**									
	16	0.86	**0.05**	**0.02**	**2601.39±33.51**	**2918.48±100.24**	**2.65**	0.76	2584.44±86.72	2537.89±124.27	-0.22
	24	**< 0.01**	**< 0.01**	**0.01**	**2416.37±160.3**	**3270.44±121.35**	**2.66**	0.08	2164.73±115.8	2458.04±94.88	1.03
	48	**0.01**	**0.01**	0.13	2926.11±83.16	2670.53±142.03	-1.25	0.5	2336.17±64.47	2459.92±164.76	0.78
unknown LC-MS	0	0.12									
	16	**0.01**	0.4	**0.01**	**47.98±3.77**	**131.09±21.02**	**9.01**	0.31	113.53±20.67	153.98±32.81	0.8
	24	0.07	0.6	0.29	77.24±7.23	97.58±15.07	1.41	0.48	95.91±12.23	77.09±22.51	-0.63
	48	0.67	0.16	**0.01**	**90.48±7.56**	**53±9.91**	**-2.02**	0.55	70.16±15.73	58.14±8.95	-0.31
unknown LC-MS	0	0.5									
	16	**< 0.01**	0.89	**< 0.01**	**1164.15±54.63**	**232.88±101.09**	**-6.96**	0.92	336.28±117.75	312.09±218.57	-0.08
	24	0.29	0.47	0.07	441.64±128.93	201.53±76.1	-0.93	0.4	679.85±228.31	414.33±195.04	-0.47
	48	0.27	0.69	0.12	456.34±178.25	144.33±35.92	-0.71	0.6	664±279.43	456.06±221.62	-0.3
unknown LC-MS	0	0.16									
	16	0.75	0.24	**< 0.01**	**5.66±0.58**	**11.51±0.74**	**4.08**	0.2	5.94±0.59	8.34±1.77	1.65
	24	**0.03**	0.16	**0.01**	**5.86±0.48**	**10.58±0.92**	**4.91**	0.94	6.27±0.73	6.42±1.8	0.09
	48	0.48	0.42	0.21	7.48±0.66	6.49±0.35	-0.6	0.28	5.68±0.48	7±1.02	1.11
unknown LC-MS	0	0.1									
	16	0.17	0.81	0.06	848.57±12.24	1282.43±145.78	14.47	0.1	767.25±52.95	1097.23±189.18	2.54

	24	0.78	0.67	0.46	1073.08±92.52	1243.92±181.06	0.92	0.57	798.18±57.51	889.92±145.56	0.65
	48	0.27	0.16	**0.04**	**908.67±83**	**1261.49±127.34**	**1.74**	0.98	1039.93±159.54	1047.18±215.47	0.02

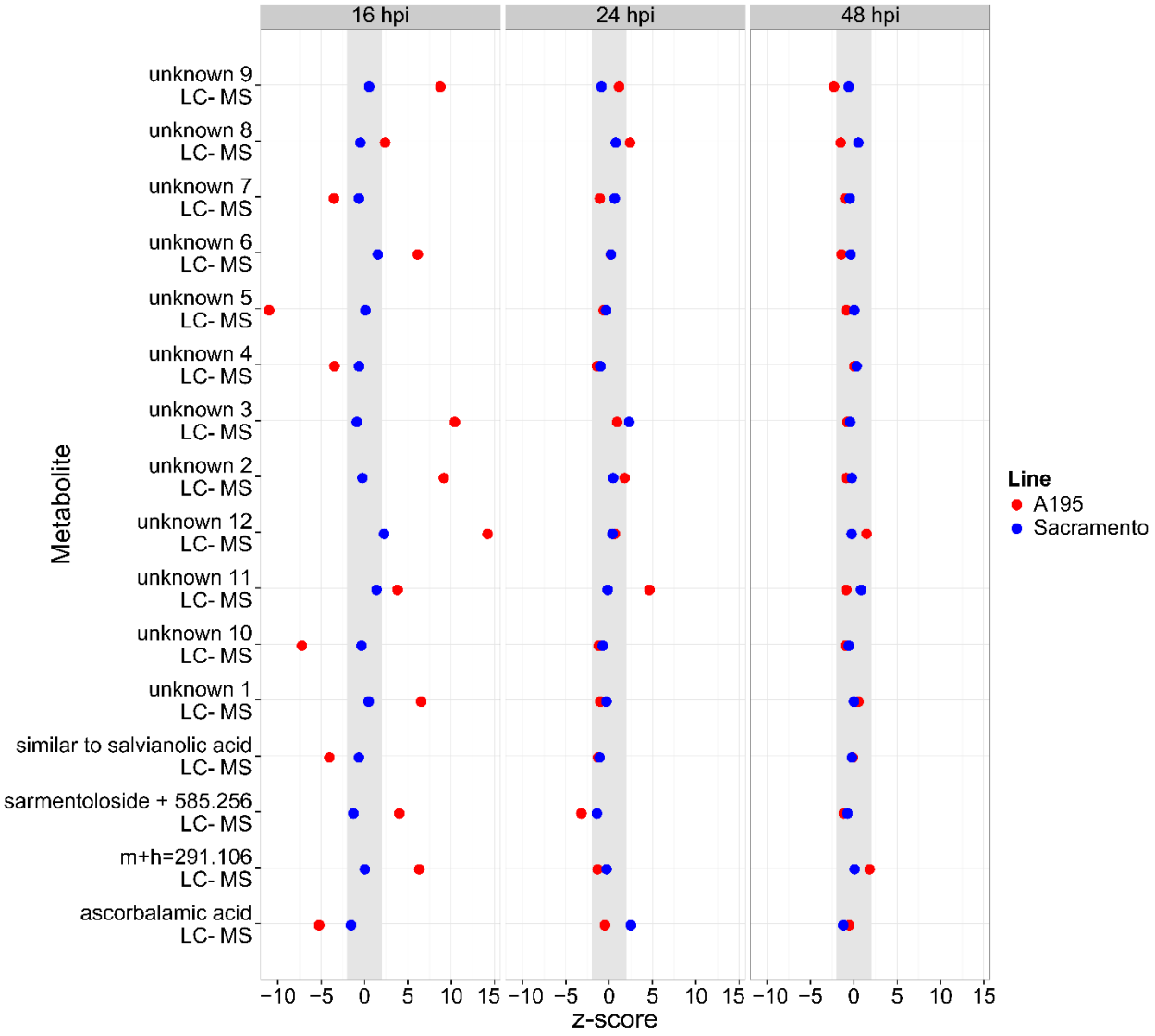

Figure 21: Z scores that illustrate the relative changes in unknown metabolite abundance at 16, 24, and 48 hpi in A195 and Sacramento when challenged with virulent inoculum relative to mock inoculum. Grey regions indicate areas of insignificant data points.

CONCLUSIONS

This research was designed to characterize metabolites and molecular mechanisms associated with resistance to *Sclerotinia sclerotiorum* in Andean common bean. Common bean lines A195 (resistant) and Sacramento (susceptible) were selected for this study because they differ in response to white mold infection. Leaves of the two bean lines were subjected to gas exchange analysis, pH analysis and both targeted and non-targeted metabolomic analysis after exposure to either mock inoculum agar plugs, the virulent strain Ss20, or the avirulent strain A4 of *S. sclerotiorum*.

A195 and Sacramento disease scores differed in the Petzoldt and Dickson straw test, where low scores indicate a higher resistance to white mold. The Straw Test scores for A195 and Sacramento were 4.9 and 6.4, respectively. These results validate the classification as Sacramento susceptible and A195 resistant, though the score in A195 is larger than previously reported.

Leaf extracts from resistant and susceptible bean lines were screened for differences in metabolite abundance in response to virulent inoculation compared to mock inoculum. A non-targeted metabolomics workflow utilized ultra-high performance liquid chromatography coupled to mass spectrometry (UPLC-MS), and gas chromatography mass spectrometry (GC-MS). A targeted workflow that utilized tandem mass spectrometry UPLC-MS/MS was also used. The relative quantities of each metabolite were compared to determine which metabolites ($p < 0.05$) differed in abundance between the resistant and susceptible lines. Metabolites that were associated with resistance were further characterized using Pathway Studio and other

metabolic databases such as SoyCyc. Pathway Studio software and SoyCYC were used to help understand biological relationships and mechanisms associated with disease resistance.

One hundred and forty metabolites were found to vary in abundance between A195 and Sacramento relative to their mock controls. The metabolites included important metabolic classes such as amines, organic acids, and sugars. Many of these metabolites were involved in important pathways associated with enhanced resistance and were involved in nitrogen remobilization, cell signaling, and secondary metabolic defenses.

This research has demonstrated that metabolic differences exist between resistant and susceptible bean lines when challenged with a virulent isolate of *Sclerotinia sclerotiorum*. The targeted and non-targeted discovery based research discovered numerous changes in the common bean metabolome and has generated enthusiasm for the discovery of physiological resistance.

S. sclerotiorum infection is a multifaceted attack by the pathogen that stimulates a multifaceted response by the host. This research demonstrated that oxalic acid (OA) is both necessary and sufficient to reduce pH in leaves to the optimal level for pathogenesis. Line A195 potentially buffers the reduction in pH induced by OA by digesting chitin with endochitinases to produce n-actylglucosamine monomers that act as a buffering agent. Our results also established that transpiration, stomatal conductance, and photosynthetic rates decreased in A195 post inoculation with virulent inoculum compared to mock inoculation or the susceptible line Sacramento. Stomatal closure may be a mechanism to slow the colonization of *Sclerotinia sclerotiorum* on the leaf surface in common bean.

Finally, Sacramento and A195 differed in metabolite abundances in response to virulent inoculation compared to mock inoculation. These metabolites were involved in important pathways associated with known enhanced resistance and are involved in nitrogen remobilization, cell signaling, and secondary metabolic defenses. Nitrogen remobilization may be important to deprive the pathogen of nitrogen for growth and the production of amino acids. By interpreting the abundance of several nitrogen containing metabolites, nitrogen remobilization appeared to be greater in the resistant line A195 than Sacramento, and led to increased levels of allantoin, asparagine, and urea. When urea is further degraded it can either be shuttled into the nitrogen recycling pathway in the plant or converted to ammonia and transported to the fungus to be integrated into amino acids [19]. Future work can include the quantitation of ammonia to better characterize the fate of urea to determine if it benefits the host or the pathogen.

Metabolites discovered in this research are potentially associated with the resistant response to Sclerotinia in common bean. Therefore, they are candidates as selection criteria in a breeding program to improve physiological resistance to white mold disease. To validate association between metabolites and the disease response, a recombinant inbred line population developed from a resistant and susceptible parent would allow for the integration of metabolite and genomic data. Recombinant inbred line populations are used to identify and map quantitative trait loci (QTL) or regions of the genome associated with resistance. Genomic mapping and identification of regions of the genome associated with resistance can also be conducted with proteins (pQTL) or single nucleotide polymorphisms (SNP). In summary, these

data support that resistance of common bean to Sclerotinia includes leaf pH regulation, stomatal closure, and metabolites that are involved in multiple interconnecting pathways.

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APPENDIX I

Table 16: Changes in secondary metabolites relative abundance at 0, 16, 24, and 48 hpi with virulent and mock inoculum in A195 and Sacramento represented as Studentized t-test, means ± se ABU, and z-scores.

			A195 vs Sacramento		A195			Sacramento				
class	Metabolite	hpi	mock	inoc	inoc vs. mock	mean mock ± se ABU	mean inoc ± se ABU	z-score	inoc vs. mock	mean mock ± se ABU	mean inoc ± se ABU	z-score
amide sugar	n-acetyl glucosamine GC-MS	0	**0.01**									
		16	0.06	0.83	**<0.01**	35.92±0.72	45.05±0.75	4.11	0.53	41.31±2.38	44.16±3.87	0.49
		24	0.44	0.22	0.11	52.54±5.375	44.48±1.12	-0.75	1	37.19±1.17	37.17±2.2	0
		48	**0.02**	**0.03**	**0.03**	57.35±3.45	47.41±1.69	-1.18	0.42	35.04±2.39	38.32±2.9	0.56
	nicotinamide GC-MS	0	**<0.01**									
		16	0.15	0.99	0.07	30.82±1.39	36.07±1.73	1.21	**0.02**	26.83±1.8	36.12±3.08	2.11
		24	0.83	0.74	0.96	30.74±1.895	30.9±2.28	0.04	0.1	26.52±1.78	30.99±1.65	1.02
		48	**0.05**	0.63	**0.01**	26.8±2.03	35.15±1.39	1.68	0.15	27.31±0.88	30.27±1.64	1.37
aromatic organic compound	hyrdoquinone GC-MS	0	0.19									
		16	0.06	0.11	**<0.01**	9.24±1.11	11.86±0.56	2.95	0.76	10.52±0.5	10.27±0.62	-0.2
		24	**0.01**	0.15	**0.05**	12.82±0.91	15.55±0.77	1.5	**0.02**	10.84±0.3	13.89±1	4.1
		48	**0.02**	0.74	0.45	12.79±0.51	11.76±1.32	-0.81	0.87	11±0.71	11.18±0.78	0.1
fatty acid	dodecanoic acid GC-MS	0	0.07									
		16	0.06	**0.02**	**<0.01**	8.84±0.72	13.73±0.77	5.35	0.94	10.31±0.58	10.24±0.8	-0.05
		24	0.32	0.06	0.81	11.87±1.18	12.24±0.94	0.16	0.84	10.06±0.21	9.85±0.98	-0.4
		48	0.35	0.32	0.61	13.67±0.98	14.92±2.35	0.52	0.42	9.99±0.37	11.43±1.69	1.6
	eicosanoic acid GC-MS	0	0.19									
		16	**<0.01**	0.82	**0.01**	9.87±1.18	12.34±0.38	1.92	**<0.01**	15.69±0.38	12.54±0.72	-3.37
		24	0.23	**<0.01**	0.19	11.45±0.6	12.56±0.48	0.93	0.17	16.43±0.65	15.02±0.71	-0.89
		48	0.36	0.13	0.98	12.9±0.87	12.86±1.12	-0.02	0.94	14.28±1.38	14.44±1.3	0.05
	glyceropalmitic acid GC-MS	0	**0.03**									
		16	0.06	0.22	**0.01**	13.48±1.09	17±0.82	2.49	0.96	14.95±0.4	15.01±1.19	0.06
		24	0.43	0.27	0.11	18.95±2.67	14.9±0.62	-0.76	0.97	14.32±0.76	14.36±0.66	0.02
		48	**0.01**	0.1	**<0.01**	16.94±1.32	28.43±2.21	3.55	**0.03**	14.2±1.55	19.05±0.93	1.28
	linolenic acid GC-MS	0	**<0.01**									
		16	**<0.01**	**0.02**	**0.05**	328.25±12.12	382.02±20.27	1.81	0.32	832.24±67.32	700.34±110.44	-0.8
		24	0.14	0.19	0.75	428.9±46.875	446.95±32.16	0.19	0.67	937.66±91.41	879.6±97.41	-0.26
		48	0.08	0.06	**<0.01**	367.62±28.23	576.26±30.49	3.02	0.47	850.49±104.25	745.47±78.23	-0.41
	palmitic acid GC-MS	0	0.15									
		16	**<0.01**	0.3	**<0.01**	970.47±85.42	1513.69±68.91	7.6	0.6	1237.9±63.02	1322.99±155.18	0.55
		24	0.56	0.08	**0.01**	1189.72±24.89	1489.27±74.43	6.02	0.52	1174.09±41.8	1242.17±93.89	0.66
		48	**0.05**	0.51	0.72	1328.55±101.46	1288.68±43.05	-0.16	0.59	1190.72±46.94	1247.09±86.57	0.49
	stearic acid GC-MS	0	0.15									
		16	**<0.01**	0.11	**<0.01**	400.73±68.64	754.32±32.62	6.46	0.84	563.28±36.42	581.76±89.31	0.21
		24	0.14	0.7	**<0.01**	530.8±12.655	716.17±36.17	7.33	0.64	535.04±18.11	555.11±36.93	0.45
		48	0.19	**<0.01**	0.23	632.68±62.11	543.8±32.87	-0.58	0.66	513.42±31.59	540.88±49.39	0.35
	apigenin, putative GC-MS	0	0.08									
		16	0.25	0.28	**<0.01**	1.29±0.4	12.55±2.38	38.4	**<0.01**	1.49±0.11	8.64±2.13	26.45
		24	**0.01**	**<0.01**	0.11	1.98±0.37	20.33±8.25	24.88	**0.01**	1.69±0.24	13.08±3.79	19.01
		48	**0.01**	**0.01**	0.07	2.38±0.24	17.62±8.14	25.36	0.08	1.79±0.21	24.16±11.51	44.26
	flavonoid conjugate LC-MS	0	**0.03**									
		16	0.89	**<0.01**	**<0.01**	6.61±0.49	26.32±2.99	16.27	0.22	6.86±1.58	10.11±1.96	0.84
		24	0.77	0.1	0.54	19.37±8.02	29.45±9.86	0.63	0.07	7.59±0.93	37.18±14.81	13.03
		48	0.15	0.59	**0.04**	14.84±1.89	132.16±59.93	25.39	**0.02**	9.58±2.01	54.61±15.86	9.13
		0	**0.01**									

flavanoid	flavonoid diglycoside LC-MS	16	0.79	0.12	**0.02**	244.11±12.89	144.85±22.18	-3.14	0.41	254.34±35	217.99±17.9	-0.42
		24	0.67	**<0.01**	**0.01**	441.17±29.735	201.14±51.16	-4.04	0.58	260.47±14.19	246.37±20.02	-0.41
		48	0.86	0.12	0.09	436.79±71.24	293.37±28.69	-0.82	0.46	309.69±44.58	270.84±12.38	-0.36
	flavonoid glucuronide LC-MS	0	**<0.01**									
		16	**<0.01**	0.33	**<0.01**	11308.95±504.85	4043.9±840.47	-5.87	0.21	5224.39±924.95	3694.58±520.48	-0.68
		24	0.16	0.98	**0.05**	12189.43±1380.99	6780.99±1634.08	-1.96	0.22	5455.32±826.39	4146.02±581.45	-0.65
		48	0.82	**0.05**	0.06	11115.07±1406.19	8014.53±560.52	-0.9	0.25	5768.03±515.77	4952.53±334.22	-0.65
	luteolin GC-MS	0	0.28									
		16	0.44	0.15	**0.02**	0.59±0.12	8.25±2.65	44.07	**<0.01**	0.69±0.1	3.42±0.8	11.4
		24	0.76	0.09	**0.05**	0.73±0.08	10.95±3.6	65.14	**0.05**	0.66±0.1	11.09±4.66	44.17
		48	0.58	0.1	**0.01**	1.11±0.16	70.5±24.13	179.56	0.1	0.83±0.14	36.82±19.77	107.09
glycerolipid ceramide	glccer(d14:1(4e)/20:0(2oh)) LC-MS	0	0.32									
		16	0.3	0.08	0.07	173.91±2.79	152.86±18.46	-3.08	0.23	160.6±11.9	182.12±11.1	0.74
		24	0.17	0.3	0.63	152.82±16	152.31±5.43	-0.02	0.8	214.13±36.62	203.76±14.07	-0.12
		48	0.18	0.53	0.43	185.83±22.41	164.33±16.77	-0.39	0.23	190.8±19.59	225.6±16.27	0.73
	laccer, putative LC-MS	0	0.12									
		16	**0.04**	0.07	**<0.01**	41.92±3.26	19.51±2.23	-2.81	0.71	29.51±3.96	27.38±3.67	-0.22
		24	0.83	**0.03**	0.14	18.72±2.485	27.04±2.63	1.67	0.14	26.08±2.59	36.32±5.87	1.62
		48	0.46	0.17	0.47	16.81±2.4	19.97±3.84	0.54	0.36	21.6±3.09	26.54±3.87	0.65
	soyacerebroside i LC-MS	0	**<0.01**									
		16	**0.01**	0.18	**0.01**	1100.48±13.87	938.58±71.68	-4.77	0.66	799.68±83.14	755.68±32.43	-0.22
		24	0.35	0.18	**0.03**	905.22±101.67	1208.24±31.34	1.49	0.46	800.74±74.08	858.09±13.04	0.32
		48	0.37	**0.03**	0.52	1133.53±102.52	1057.4±59.01	-0.3	0.29	756.82±52.08	864.55±76.26	0.84
hormones	gibberellin a37 glucosyl ester, putative LC-MS	0	0.63									
		16	**0.01**	0.7	**0.05**	14.58±1.2	39.52±9.81	8.53	0.65	23.96±2.87	29.93±13.6	0.85
		24	**<0.01**	**0.02**	0.07	52.44±21.1	19.78±4.34	-0.77	0.25	28.74±4.99	66.78±30.66	3.11
		48	0.42	0.38	0.73	34.16±5.78	30.58±5.59	-0.25	0.51	28.75±7.09	55.85±39.64	1.56
	methyl 7-epi-12-hydroxyjasmonate glucoside, putative LC-MS	0	**0.01**									
		16	**0.02**	0.09	0.46	263.04±13.17	146.94±62.87	-3.6	**0.02**	174.41±28.41	77.49±13.53	-1.39
		24	**<0.01**	**<0.01**	0.27	163.67±25.48	252.76±38.03	1.75	0.07	102.63±7.58	153.88±24.12	2.76
		48	**<0.01**	0.07	0.16	225.55±36.18	157.58±32.79	-0.77	0.51	186.01±72.06	128.63±24.52	-0.33
	isopentenyl diphosphate LC-MS	0	0.59									
		16	0.27	**<0.01**	**0.01**	6.39±0.6	9.94±0.77	2.43	0.71	4.89±1.13	4.39±0.34	-0.18
		24	**0.02**	0.76	0.22	7.36±1.17	11.55±1.81	1.79	**0.01**	4.26±0.5	7.88±0.99	2.94
		48	0.31	0.26	0.82	9.48±0.55	9.81±1.48	0.25	0.25	9.6±1.27	7.83±0.36	-0.57
	lipid LC-MS	0	0.09									
		16	**0.05**	0.11	**0.01**	412.98±13.1	317.77±38.27	-2.97	0.14	304.23±48.02	459.93±88.3	1.32
		24	0.51	0.21	0.83	319.75±41.535	291.49±48.95	-0.34	**0.04**	286.99±17.84	380.89±35.63	2.15
		48	0.12	0.1	0.83	216.75±20.8	229.94±69.51	0.26	0.6	329.27±36.22	296.36±46.65	-0.37
	multiple lipids LC-MS	0	**0.01**									
		16	**<0.01**	0.16	**0.01**	2.66±0.24	4.22±0.27	2.67	0.14	6.68±0.79	5.03±0.58	-0.85
		24	0.3	0.63	0.2	2.85±0.255	3.31±0.3	0.91	0.9	6.81±0.6	6.68±0.74	-0.08
		48	0.4	0.71	0.58	3.13±0.28	3.35±0.29	0.31	0.91	6.2±0.71	6.06±0.83	-0.08
	multiple lipids LC-MS	0	0.95									
		16	0.22	0.95	**<0.01**	48.31±3.27	89.92±9.12	5.19	0.18	61.52±9.5	88.42±16.82	1.16
		24	**0.04**	0.21	0.12	57.57±8.725	71.94±5.76	0.82	0.98	54.27±9.83	54.77±13.31	0.02
		48	0.07	0.71	0.66	49.67±8.16	45.07±6.74	-0.23	0.65	54.79±11.46	48.11±6.7	-0.24
	multiple lipids	0	0.19									
		16	0.58	0.19	**0.01**	10.44±0.64	15.44±1.13	3.21	0.19	9.6±1.29	12.35±1.41	0.87

	LC-MS	24	0.26	0.09	0.96	16.09±5.63	10.79±0.69	-0.47	0.52	8.81±0.64	10.01±1.69	0.77
		48	0.89	0.61	0.08	9.73±1.07	7.32±0.71	-0.91	0.83	8.8±1.02	8.51±0.65	-0.11
		0	0.44									
lipid	multiple lipids	16	0.2	0.85	**<0.01**	15.47±0.62	22±0.93	4.26	0.3	18.3±1.96	21.56±2.25	0.68
	LC-MS	24	0.3	0.07	0.1	15.9±2.31	20.27±1.23	0.95	0.46	17.29±1.45	19.11±1.87	0.51
		48	**<0.01**	0.18	**0.05**	16.28±1.21	12.99±1.06	-1.11	0.95	16.53±1.84	16.39±0.93	-0.03
		0	**<0.01**									
	multiple lipids	16	**<0.01**	0.07	**<0.01**	20.78±0.56	31.14±2.4	7.62	0.07	30.19±2.44	36.44±1.5	1.05
	LC-MS	24	**0.03**	**0.03**	0.06	20.11±3.13	24.78±0.78	0.75	0.18	29.76±1.65	26.3±1.77	-0.85
		48	0.12	0.21	0.99	18.31±1.89	18.27±1.66	-0.01	0.67	26.86±3.18	24.91±2.6	-0.25
		0	0.1									
	multiple lipids	16	0.76	0.4	**0.01**	24.51±1.74	46.79±6.47	5.23	0.3	25.97±4.35	35.81±8.35	0.92
	LC-MS	24	0.89	0.06	0.2	32.68±6.83	31.9±2.59	-0.06	0.98	24.72±3.36	24.55±5.93	-0.02
		48	0.19	0.6	**0.01**	25.84±2.11	17.62±1.71	-1.59	0.86	21.02±3.23	20.13±3.46	-0.11
		0	0.42									
	multiple lipids	16	0.51	0.88	**<0.01**	8.75±0.47	12±0.67	2.82	0.08	9.32±0.7	12.32±1.45	1.75
	LC-MS	24	0.08	0.56	0.58	11.22±2.045	10.08±0.25	-0.28	0.25	8.81±0.69	10.6±1.29	1.06
		48	0.21	**<0.01**	**0.03**	10.23±0.69	7.93±0.69	-1.35	0.93	8.36±0.73	8.45±0.62	0.05
		0	0.1									
	multiple lipids	16	**<0.01**	0.56	**0.01**	6.77±0.37	9.45±0.67	2.99	0.25	11.7±1.29	9.76±0.7	-0.61
	LC-MS	24	0.37	0.18	**0.04**	6.93±1.27	8.73±0.51	0.71	0.47	12.39±1.37	14.32±2.17	0.58
		48	0.68	0.98	0.15	7.7±0.31	7±0.32	-0.94	0.69	11.1±1.6	12.1±1.62	0.25
		0	0.4									
	multiple lipids	16	0.12	0.58	**0.02**	499.52±45.23	947.52±157.23	4.81	0.32	800.49±173.26	1134.39±275.43	0.79
	LC-MS	24	0.31	0.16	0.46	589.66±168.17	748.32±135.15	0.47	0.97	658.63±154.8	649.66±175.79	-0.02
		48	0.96	0.93	0.35	414.7±121.39	279.6±69.38	-0.45	0.35	646.32±154.95	457.16±91.77	-0.5
		0	0.68									
organic	urea	16	0.16	0.39	**0.01**	88.64±49.03	362.41±82.45	48.04	0.25	168.25±51.94	264.4±58.73	0.76
	GC-MS	24	0.1	0.29	**0.03**	452.91±154.065	132.25±20.31	-1.04	0.1	362±137.98	106.26±18.76	-0.76
		48	0.95	0.74	0.74	604.49±150.24	685.27±194.77	0.22	0.2	200.02±71.82	407.86±129.08	1.18
		0	0.11									
	hyaluronan, putative	16	0.77	0.14	**<0.01**	9.4±0.31	4.36±0.24	-6.59	0.41	8.89±1.7	6.91±1.4	-0.47
other	LC-MS	24	0.77	0.08	**0.03**	13.01±3.75	4.75±0.35	-1.1	0.61	9.06±0.69	8.25±1.39	-0.48
		48	0.16	0.73	0.7	10.34±1.36	11±1.06	0.2	0.59	11.5±1.77	10.18±1.36	-0.3
		0	**0.03**									
	thymol glucoside	16	**0.04**	0.12	**<0.01**	12.92±1.14	19.48±0.58	7.23	0.42	14.75±0.71	16.23±1.76	0.86
	GC-MS	24	0.46	0.11	**0.01**	16.39±0.285	20.48±0.91	7.22	0.27	13.69±0.3	15.56±1.56	2.54
		48	**0.01**	**0.04**	**0.03**	18.91±1.57	36.05±7.03	4.46	**0.04**	14.91±0.74	17.8±0.92	1.59
		0	0.66									
peptide	peptide	16	**0.03**	0.06	**0.03**	3.6±0.08	5.52±0.54	9.68	0.09	5.15±0.6	6.5±0.29	0.91
	LC-MS	24	0.24	**0.03**	0.12	4.28±0.655	4.74±0.4	0.35	0.3	5.58±0.44	4.64±0.73	-0.87
		48	0.61	0.64	0.75	5±0.58	5.24±0.38	0.17	0.85	5.92±0.9	6.13±0.38	0.09
		0	0.22									
purine	guanosine	16	**<0.01**	0.6	**<0.01**	143.38±3.16	45.73±10.1	-12.62	0.47	49.94±9.81	66.32±20.74	0.68
	LC-MS	24	0.38	0.08	0.1	68.36±23.86	44.85±5.52	-0.49	**0.02**	65.51±12.21	126.22±16.76	2.03
		48	**0.02**	0.35	0.59	77.01±18.89	64.69±12.2	-0.27	0.18	144.61±35.01	84.22±15.71	-0.7
		0	**<0.01**									
	coumestrol	16	0.06	0.17	0.21	5.89±1.39	19.42±8.24	3.98	0.49	2.94±0.25	3.35±0.55	0.66
	LC-MS	24	0.42	**<0.01**	0.13	6.11±2.135	47.04±15.95	9.59	0.23	3.32±0.61	8.12±3.74	3.19

		48	**0.01**	0.07	**<0.01**	6.46±0.52	155.36±41.51	117.3	0.07	4.22±1.17	14.48±4.82	3.59
	kievitone LC-MS	0	0.97									
		16	0.21	0.22	0.25	8.19±2.6	34.98±17.44	4.21	0.57	4.63±0.6	4.21±0.33	-0.29
		24	**0.04**	**<0.01**	0.09	4.73±0.53	88.85±28.86	79.15	0.08	4.64±0.86	46.46±21.78	19.87
		48	**0.01**	**0.01**	**0.01**	9.69±2.06	610.71±229.29	118.97	0.2	7.04±2.36	198.76±141.09	33.11
	phaseolin/abyssinone i LC-MS	0	0.79									
		16	0.14	0.14	0.14	12.15±3.13	68.19±27.44	7.31	0.46	7.06±0.69	8.4±1.74	0.79
		24	0.14	0.23	0.08	11.44±3.82	137.48±42.39	16.49	0.07	7.8±1.7	20.8±6.16	3.11
		48	0.35	0.82	**<0.01**	17.46±3.2	617.75±108.76	76.59	**0.04**	11.39±4.16	105.43±39.96	9.22
secondary metabolite	phaseollidin/phaseollin isoflavan LC-MS	0	0.15									
		16	0.11	0.34	0.06	49.22±8.83	78.81±25.15	1.37	0.65	85.14±18.74	73.2±16.11	-0.26
		24	0.28	0.18	0.33	69.51±13.025	118.6±24.26	1.88	0.25	103.04±28.96	66.15±9.03	-0.52
		48	0.12	**0.02**	0.44	84.55±19.6	103.3±12.71	0.39	0.16	56.1±9.5	80.86±12.15	1.06
	quinic-acid like compound LC-MS	0	0.22									
		16	0.21	0.13	**0.05**	699.57±30.64	978.86±122.58	4.17	0.24	1003.56±225.28	1402.42±213.6	0.72
		24	**<0.01**	0.16	0.15	930.1±146.46	603.99±100.79	-1.11	0.45	731.6±113.92	879.09±149.58	0.53
		48	**0.01**	**<0.01**	0.18	744.95±163.49	1083.8±181.72	0.85	0.18	754.18±80.81	1110.3±229.16	1.8
	soyasaponin i LC-MS	0	0.29									
		16	0.06	**0.01**	**0.01**	28.84±1.92	59.72±9.82	6.57	**0.04**	58.51±14.06	20.68±3.1	-1.1
		24	**0.03**	**0.05**	**0.05**	84.35±22.28	28.46±5.35	-1.25	**<0.01**	70.08±4.65	41.64±5.22	-2.5
		48	0.63	0.47	0.09	83.11±6.54	125.44±24.89	2.64	0.14	39.42±12.39	65.98±9.35	0.88
	unknown LC-MS	0	0.32									
		16	0.28	**<0.01**	**<0.01**	39.18±0.75	122.35±16.09	45.09	0.1	33.6±4.85	46.17±4.56	1.06
		24	**0.05**	0.07	0.54	87.65±25.49	107.38±12.25	0.39	**<0.01**	40.1±8.21	98.45±11	2.9
		48	0.78	**0.01**	**0.05**	60.67±10.13	157.81±24.14	3.92	**<0.01**	54.34±10.37	239.11±27.38	7.27
sterol	aldosterone, putative GC-MS	0	**<0.01**									
		16	0.61	0.23	0.15	0.27±0.02	1.83±1	20.75	0.1	0.25±0.02	0.41±0.09	2.56
		24	0.25	0.19	0.18	0.38±0.065	3.24±1.57	22.38	0.22	0.31±0.06	1.55±0.96	8.48
		48	0.08	**0.02**	**0.05**	0.45±0.11	31.41±15.47	112.05	0.12	0.24±0.02	2.42±1.27	34.25
	beta-sitosterol GC-MS	0	**<0.01**									
		16	**0.01**	0.31	**<0.01**	177.46±12.25	226.08±5.02	2.93	0.71	205.11±4.63	210.25±13.48	0.45
		24	0.1	0.4	0.39	204.54±3.885	218.71±12.18	1.82	0.44	194.55±6.84	182.32±13.45	-0.73
		48	0.35	0.18	0.31	266.12±17.41	242.39±15.2	-0.56	0.16	187.9±6.93	212.21±14.22	1.43
	stigmasterol GC-MS	0	0.12									
		16	**0.04**	0.11	**<0.01**	522.66±78.58	957.08±39.58	7.01	0.75	673.29±58.01	715.81±127.3	0.3
		24	**<0.01**	0.43	**0.02**	670.96±37.215	877.93±49.77	2.78	0.31	598.15±41	681.11±65.92	0.83
		48	0.16	0.47	0.44	795.95±83.22	720.55±45.62	-0.37	0.42	632.06±43.43	705.28±71.84	0.69
	c5 hexose GC-MS	0	0.2									
		16	0.17	0.93	**<0.01**	284.03±24.19	230.92±6.44	-2.75	0.11	264.93±10.33	229.05±18.58	-1.42
		24	0.23	**0.01**	0.22	280.66±30.1	240.15±15.5	-0.67	0.39	236.38±12.59	221.4±10.87	-0.49
		48	0.87	**0.02**	0.9	307.87±14.41	302.57±40.07	-0.15	0.08	207.13±14.63	250.62±14.8	1.21
	c5 sugar acid GC-MS	0	**0.04**									
		16	**0.02**	0.14	**<0.01**	60.25±3.94	41.24±2.63	-2.61	0.58	47.95±3.38	51.46±5.47	0.42
		24	0.43	0.17	0.1	53.87±3.515	43.29±4.02	-1.5	0.77	46.64±2.57	45.35±3.53	-0.21
		48	**0.02**	0.42	0.34	63.54±3.89	71.91±8.09	0.88	**0.04**	44.98±4.41	58.96±3.41	1.29
	c6 sugar GC-MS	0	0.54									
		16	**<0.01**	0.61	**0.05**	5128.41±161.65	3194.6±858.44	-17.64	**<0.01**	4691.96±70.05	2637.53±495.42	-11.97
		24	0.09	0.18	0.98	3568.76±498.385	3588.26±471.39	0.02	0.98	4034.08±397.78	4054.9±649.3	0.02

		48	0.25	0.25	0.16	2534.94±677.7	1371.38±412.98	-0.7	0.45	3491.53±420	3005.16±413.87	-0.47
		0	0.45									
	c6 sugar	16	**0.01**	0.65	**<0.01**	78.52±3.3	45.19±5.54	**-2.48**	**0.05**	57.05±3.28	40.73±7.08	**-2.03**
	GC-MS	24	0.45	**0.01**	0.24	60.19±6.175	49.36±5.59	-0.88	0.82	50.98±5.1	49.24±5.5	-0.14
		48	0.16	0.3	0.16	52.77±9.43	35.92±6.32	-0.73	0.2	56.79±7.21	42.82±6.36	-0.79
		0	0.2									
	c6 sugar pyranose	16	0.06	0.71	**0.02**	1565.75±53.62	848.74±243.37	-2.96	**0.01**	1303.68±76.41	723.62±181.59	-3.1
	GC-MS	24	0.87	0.23	0.95	807.88±187.03	821.7±128.7	0.04	0.76	1149.81±133.47	1081.86±170.9	-0.21
		48	**0.02**	0.57	0.18	521.97±129.32	307.61±77.82	-0.68	0.96	773.22±127.96	762.42±154.06	-0.03
		0	**0.02**									
	cyclic sugar alcohol	16	**0.04**	0.79	**<0.01**	158.93±13.4	109.14±11.87	-4.52	0.37	128.45±12.49	113.45±8.91	-0.49
	GC-MS	24	0.6	**<0.01**	**<0.01**	235.05±27.32	102.4±11.19	-2.43	0.12	129.96±10.74	105.49±9.29	-0.93
		48	0.08	0.13	**0.01**	168.74±8.64	225.65±18.79	2.69	0.45	106.26±14.96	125±17.16	0.51
		0	0.68									
	disaccharide	16	0.14	0.7	0.13	325.85±41.26	437.96±67.41	**3.97**	0.09	357.54±16.04	477.35±67.77	**3.05**
	GC-MS	24	0.4	0.07	**0.03**	588.11±124.425	304.59±37.94	**-1.14**	0.51	303.1±11.52	317.68±18.06	0.52
		48	0.22	0.26	**0.01**	272.19±35.57	560.06±92.94	3.3	0.71	363.47±76.83	409.24±85.41	0.24
		0	0.08									
	dissacharide	16	0.29	0.08	**0.05**	1263.98±95.42	874.97±75.76	-1.13	0.2	1610.88±276.03	1160.01±114.36	-0.67
	GC-MS	24	**0.02**	0.11	0.08	800.21±132.375	1116.92±92.18	1.2	0.65	1972.33±205.02	2172.26±378.96	0.4
		48	0.93	0.21	**0.03**	843.15±82.85	1235.66±145.7	1.93	0.57	1990.78±278.39	1728.08±332.87	-0.39
		0	0.08									
	erythronic acid lactone	16	**0.04**	**0.05**	0.08	14.45±0.67	11.31±0.78	**-1.01**	**0.02**	18.56±1.16	14.35±0.98	**-1.48**
	GC-MS	24	**<0.01**	**<0.01**	0.14	12.76±1.145	9.65±1.31	**-1.36**	**<0.01**	17.79±0.85	12.02±0.79	**-2.78**
		48	**<0.01**	**0.01**	**0.01**	17.56±1.42	11.79±0.99	-1.66	0.94	12.13±1.41	12.26±0.91	0.04
		0	**<0.01**									
	fructose	16	0.25	0.52	**<0.01**	13617.2±742.24	7258.69±1567.44	-9.8	0.11	12442.83±924.66	8963.64±1880.52	-1.54
	GC-MS	24	1	0.8	0.61	6750.18±3233.065	8595.73±1841.41	0.29	0.44	9880.64±2338.44	12525.61±2328.29	0.46
		48	0.4	0.24	0.14	4408.76±1165.36	2180.44±830.86	-0.78	0.16	11063.65±2009.32	6909.95±1631.44	-0.84
		0	0.19									
	glucopyranose	16	**0.02**	**<0.01**	**0.01**	393.42±11.64	706.9±100.16	7.82	0.44	235.41±52.47	187.2±15.97	-0.38
	GC-MS	24	0.22	0.7	0.07	456.3±98.29	797.24±112.64	1.73	**0.01**	298.37±59.11	694±110.1	2.73
		48	0.09	0.56	0.08	392.19±82.38	830.84±233.14	2.17	**<0.01**	212.38±27.28	885.26±54.77	10.07
		0	0.34									
	glucose	16	0.42	0.47	0.2	11670.51±197.95	9722.5±1400.83	-4.71	**<0.01**	11359.33±330.57	8461.6±619.31	-3.58
	GC-MS	24	0.1	0.7	0.2	9435.22±829.335	10978.65±717.47	0.93	0.63	10444.32±859.24	9856.36±808.4	-0.28
		48	0.29	0.18	0.15	7880.74±1489.49	5044.81±1180.49	-0.78	0.57	9400.97±666.49	8781.25±762.52	-0.38
		0	0.24									
	sedoheptulose	16	0.14	**0.04**	0.46	262.93±23.39	250.45±13.23	-0.6	0.94	294.85±17.99	296.53±11.8	0.04
	GC-MS	24	**0.02**	**0.03**	**0.03**	290.08±37.935	204.38±10.46	-1.13	0.79	251.73±15.62	245.4±16.65	-0.17
		48	0.06	**0.01**	0.59	257.96±17.2	275.34±29.06	0.41	0.78	232.65±18.98	240.12±15.53	0.16
		0	**0.03**									
	sucrose	16	0.63	0.26	0.09	11112.03±1023.09	9153.15±994.31	-3.28	0.89	11415.36±561.3	11230.98±1303.31	-0.13
	GC-MS	24	0.15	0.68	0.06	8161.66±460.39	6272.26±643.66	-2.05	0.55	11000.52±893.92	12350.32±1964.13	0.62
		48	0.55	0.23	0.25	8241.46±385.44	7211.88±818.24	-1.09	0.31	12354±1535.16	9557.38±1985.77	-0.74
		0	0.41									
	sugar	16	0.08	0.06	**0.01**	1803.33±130.84	1097.9±44.17	-1.57	0.09	1310.05±166.68	957.27±42.8	-0.86
sugar	GC-MS	24	0.61	0.61	0.06	1108.05±80.325	1524.1±141.62	2.59	0.51	1616.16±155.19	1862.77±325.53	0.65
		48	0.91	**0.03**	0.13	1041.85±38.56	1343.75±194.35	3.2	0.23	1839.82±253.18	1403.11±191.82	-0.7

	0	**<0.01**									
sugar	16	**0.01**	**<0.01**	**0.02**	56.06±5.34	78.01±7.77	5.52	0.12	110.65±18.25	151.96±14.7	0.92
GC-MS	24	0.82	0.26	**0.03**	124.99±29.215	60.98±2.95	-1.1	0.78	110.66±16.7	117.04±14.61	0.16
	48	0.72	0.76	**<0.01**	91.79±14.16	166.12±12.51	2.14	0.32	109.15±22.92	146.9±26	0.67
	0	0.06									
sugar	16	**0.01**	**0.02**	0.2	232.15±44.32	203.01±21	-2.75	0.77	294.44±20.73	303.95±23.61	0.19
GC-MS	24	0.94	0.27	**0.01**	412.55±87.9	151.39±8.23	-1.49	0.15	291.55±28.4	225.72±31.47	-0.95
	48	0.42	0.14	0.12	350.91±46	448.51±36.96	0.87	0.46	260.05±52.21	315.52±42.66	0.43
	0	**0.01**									
sugar	16	**0.02**	**<0.01**	0.53	128.17±14.1	121.33±9.71	-0.73	0.09	187.91±20.93	245.93±21.43	1.13
GC-MS	24	**<0.01**	**<0.01**	**0.03**	215.51±52.08	97.55±9.76	-1.13	**0.01**	140.15±10.89	185.47±9.18	1.7
	48	**<0.01**	**<0.01**	**<0.01**	134.54±19.8	240.08±23.43	2.18	0.34	156.59±21.6	195.8±31.4	0.74
	0	0.44									
sugar	16	**0.03**	0.65	**0.01**	1176.93±64.57	1573.43±89.74	2.3	0.37	1507.15±104.29	1627.12±60.19	0.47
GC-MS	24	0.69	0.07	0.12	1609.27±192.225	1320.38±56.81	-0.75	0.61	1352.45±137.49	1267.66±88.11	-0.25
	48	0.18	0.21	0.17	1464.65±104.79	1288.21±63.21	-0.69	0.64	1323.45±121.47	1243.15±100.43	-0.27
	0	0.81									
sugar	16	0.1	0.85	**0.02**	106.49±11.44	184.04±27.57	7.73	0.25	139.06±17.4	176.12±25.76	0.87
GC-MS	24	0.28	0.28	0.14	160.62±33.25	110.71±12.08	-0.75	0.55	104.04±12.73	114.16±10.38	0.32
	48	0.24	0.52	0.09	102.39±11.07	139.23±17.93	1.36	0.65	119.32±8.44	110.88±15.86	-0.41
	0	0.44									
sugar	16	0.42	0.47	**0.01**	248.26±21.03	170.62±20.22	-4.79	0.91	189.75±69.39	199.7±31.78	0.06
GC-MS	24	0.69	0.07	**<0.01**	391.37±84.42	96.6±11.99	-1.75	0.37	305.64±93.14	194.82±71.41	-0.49
	48	**0.05**	0.65	**0.02**	164.12±21.98	422.57±100.32	4.8	0.46	190.55±83.51	283.03±75.75	0.45
	0	**0.02**									
sugar	16	0.69	0.23	**<0.01**	513.73±239.04	157.07±26.64	-5.36	0.38	458.31±133.64	297.92±103.3	-0.49
GC-MS	24	0.17	**0.04**	**<0.01**	804.91±228.345	54±9.33	-1.64	0.1	504.23±118.75	235.57±90.53	-0.92
	48	0.11	**0.01**	0.82	236.96±110.3	208.8±58.91	-0.1	0.08	197.05±35.13	108.63±22.3	-1.03
	0	0.81									
sugar (cellobiose)	16	0.73	0.49	0.62	1180.01±183.36	1024.16±258.26	-0.45	0.28	1089.31±209.1	806.74±101.16	-0.55
GC-MS	24	0.26	0.24	0.47	855.32±237.43	693.1±84.54	-0.34	0.61	1148.72±237.46	976.92±230.12	-0.3
	48	0.14	0.96	0.28	623.66±105.41	755.02±47.31	0.51	**0.02**	1282.25±197.47	600.05±71.06	-1.41
	0	0.46									
sugar (pyranose)	16	0.06	0.16	0.14	770.16±172.51	1057.84±174.56	3.71	**0.01**	945.35±75.4	1441.08±154.96	2.68
GC-MS	24	**<0.01**	**<0.01**	**0.02**	1944.16±461.165	800.54±102.15	-1.24	0.12	935.99±71.09	1068.51±33.33	0.76
	48	**0.01**	**<0.01**	**<0.01**	790.44±130.18	1937.97±301.51	3.6	0.86	1294.93±264.46	1370.79±286.8	0.12
	0	0.39									
sugar (pyranose)	16	0.67	0.86	**0.01**	384.4±37	126.61±28.83	-1.68	**0.04**	344.79±64.13	137.34±51.52	-1.32
GC-MS	24	0.28	0.36	0.93	427.13±227.27	405.54±118.92	-0.05	0.35	176.35±62.72	269.35±70.44	0.61
	48	0.91	0.36	0.65	178.46±41.09	226.59±102.2	0.48	**0.05**	447.43±127.43	125.36±34.54	-1.03
	0	0.62									
threose	16	0.51	0.1	**<0.01**	82±14.08	38.04±2.13	-4.17	0.11	92.08±14.04	59.18±10.93	-0.96
GC-MS	24	0.08	**<0.01**	**0.01**	63.34±4.035	39.04±4.51	-3.01	0.2	64.48±9.04	91.74±17.42	1.23
	48	0.59	0.11	0.59	47.03±2.22	42.62±8.38	-0.81	0.14	83.14±17.13	51.08±6.14	-0.76
	0	0.58									
unknown disaccharide	16	**0.03**	0.61	**<0.01**	385.8±12.31	177.3±41.4	-4.24	**0.04**	278.25±39.14	146.82±35.6	-1.37
GC-MS	24	0.55	0.09	0.62	232.58±51.345	201.41±35.39	-0.3	0.8	245.02±42.7	261.31±45.12	0.16
	48	0.31	0.14	0.43	173.13±42.64	134.31±21.12	-0.37	0.76	205.7±33.14	189.34±36.78	-0.2
	0	0.55									

	unknown, putative sugar	16	0.33	**<0.01**	0.86	1825.86±186.55	1804.76±17.55	-0.08	0.09	1992.88±125.51	2299.27±95.24	1
	GC-MS	24	0.35	**0.02**	**0.04**	2206.72±210.095	1698.72±90.65	-1.21	0.97	1866.32±98.3	1860.93±70.47	-0.02
		48	0.27	**0.02**	**<0.01**	1620.04±155.58	2531.18±94.73	2.39	0.46	2050.93±152.91	2208.84±119.42	0.42
		0	**0.03**									
	xylulose	16	0.18	0.1	0.28	572.3±401.17	989.37±348.69	1.73	0.28	1326.64±520.11	2182.19±521.3	0.67
	GC-MS	24	**0.02**	0.45	**0.01**	3093.94±1071.845	106.57±38.93	-1.39	0.29	1854.01±740.71	844.97±514.94	-0.56
		48	**0.04**	0.06	**0.01**	1819.69±458.18	4047.58±551.96	1.99	0.3	1115.32±599.26	2070.35±576.89	0.65
		0	**0.01**									
terpenoid pathway	nerolidol tri-glycoside	16	**<0.01**	0.44	0.47	779.68±85.13	896.42±123.22	0.78	**0.03**	1930.95±94.19	1165.17±305.97	-3.32
	LC-MS	24	0.22	0.16	0.14	814.56±133.14	495.44±74.17	-1.2	0.66	1980.66±187.79	1820.8±304.09	-0.35
		48	0.57	0.55	0.85	642.36±63.45	665.15±105.79	0.15	0.9	1474.59±260.7	1523.47±257.34	0.08
		0	0.11									
	ascorbalamic acid	16	0.15	0.68	**<0.01**	152.96±7.09	66.69±4.92	-4.97	0.08	126.99±15.07	79.31±19.1	-1.29
	LC-MS	24	**0.01**	**0.01**	0.71	76.27±14.655	69.89±10.92	-0.22	0.17	93.66±11.66	172.76±52.17	2.77
		48	**<0.01**	**0.02**	0.7	50.17±6.12	46.12±9.87	-0.27	0.11	152.23±34.9	71.34±24.52	-0.95
		0	0.3									
	m+h=291.106	16	**0.01**	**0.03**	**0.05**	23.96±0.6	33.66±3.49	6.57	0.54	40.12±4.5	43.57±2.47	0.31
	LC-MS	24	1	**<0.01**	**0.05**	97.7±36.095	19.65±1.35	-1.08	0.95	38.88±5.57	38.45±4.38	-0.03
		48	**<0.01**	0.65	**0.01**	39.16±7.17	75.66±9.7	2.08	0.57	45.36±8.6	52.88±8.7	0.36
		0	0.08									
	sarmentoloside + 585.256	16	**0.01**	0.21	**<0.01**	32.4±5.26	87.52±10.72	4.28	**0.05**	321.67±82.24	115.69±18.36	-1.02
	LC-MS	24	0.1	0.11	0.09	50.93±2.455	36.49±4.37	-2.94	**0.03**	510.39±95.17	243.1±44.51	-1.15
		48	**0.01**	0.26	0.09	46.5±4.87	35.79±3.77	-0.9	0.42	153.15±25.47	124.61±19.04	-0.46
		0	0.96									
	similar to salvianolic acid	16	0.17	0.89	**0.01**	643.31±25.32	408.29±51.51	-3.79	0.64	537.56±66.38	476.33±113.98	-0.38
	LC-MS	24	0.66	**<0.01**	**0.02**	1031.81±356.23	298.52±16.61	-1.03	0.23	673.25±98.05	474.45±119.83	-0.83
		48	0.53	0.52	0.75	924.92±135.29	979.25±118.22	0.16	0.91	620.78±114.71	635.87±40.57	0.05
		0	0.08									
	unknown	16	**0.01**	**<0.01**	**<0.01**	8.68±0.33	14.2±0.85	6.81	0.18	16.32±2.14	20.23±1.34	0.74
	LC-MS	24	**0.03**	0.1	0.2	12.91±2.47	9.2±0.42	-0.75	0.94	15.29±2.58	14.99±3.08	-0.05
		48	0.57	0.19	0.13	11.21±1.08	13.3±0.67	0.79	0.58	13.9±2.26	15.48±1.16	0.29
		0	**0.05**									
	unknown	16	**<0.01**	0.18	**<0.01**	2.95±0.25	8.74±1.25	9.43	0.95	11.91±1.94	12.07±1.58	0.03
	LC-MS	24	0.54	0.93	0.18	4.45±1.985	12.59±3.11	2.05	0.42	13.71±1.67	16.66±3.09	0.72
		48	0.52	0.08	0.29	14.42±2.99	9.87±3.38	-0.62	0.98	20.66±3.12	20.86±6.37	0.03
		0	0.32									
	unknown	16	**0.02**	**0.03**	**0.01**	20.94±1.95	72.04±15.64	10.69	0.28	36.87±5.65	28.32±4.53	-0.62
	LC-MS	24	0.23	**<0.01**	0.33	63.48±19.675	110.1±20.8	1.18	0.14	52.18±10.94	120.72±41.8	2.56
		48	**<0.01**	0.36	0.35	56.38±9.11	45.34±6.56	-0.49	0.77	41.79±10.57	37.58±7.2	-0.16
		0	0.96									
	unknown	16	0.73	0.63	**<0.01**	254.57±10.28	173.84±14.27	-3.21	0.66	242.42±32.57	213.61±56.68	-0.36
	LC-MS	24	0.6	0.82	**0.02**	487.13±163.81	115.67±8.86	-1.13	0.26	305.92±54.82	206.34±63.47	-0.74
unknown		48	0.32	0.47	0.52	409.55±77.11	471.75±61.97	0.33	0.28	246.4±35.52	297.44±20.03	0.59
		0	0.09									
	unknown	16	**<0.01**	0.45	**<0.01**	63.91±1.6	21.76±5.85	-10.74	0.7	31.55±4.05	35.2±8.81	0.37
	LC-MS	24	**0.01**	0.38	0.11	34.52±13.32	25±1.58	-0.36	0.89	35.76±5.46	34.69±5.51	-0.08
		48	0.18	0.15	0.45	36.87±5.17	29.43±2.22	-0.59	0.6	43.23±9.3	50.51±8.48	0.32
		0	0.11									
	unknown	16	0.98	0.84	**0.02**	30.49±1.35	51.7±6.07	6.4	0.11	30.6±4.45	50.19±11.12	1.8

	LC-MS	24	0.1	0.67	0.12	32.46±5.44	37.34±2.38	0.45	0.75	26.36±2.39	29.18±8.15	0.48
		48	0.09	0.2	0.06	29.34±2.36	22.62±2.23	-1.16	0.88	28.74±4.95	27.59±4.7	-0.09
		0	0.83									
	unknown	16	0.08	0.83	**0.01**	18.72±0.91	11.43±1.51	-3.26	0.51	14.1±2.22	12.01±1.98	-0.38
	LC-MS	24	0.12	0.91	**0.04**	17.97±3.98	11.49±0.49	-0.81	0.55	11.98±0.51	13.09±1.74	0.89
		48	0.41	0.17	0.32	16.73±0.98	14.97±1.44	-0.73	0.69	13.81±2.81	12.5±0.8	-0.19
		0	**0.02**									
	unknown	16	0.86	**0.05**	**0.02**	2601.39±33.51	2918.48±100.24	2.65	0.76	2584.44±86.72	2537.89±124.27	-0.22
	LC-MS	24	**<0.01**	**<0.01**	**0.01**	2416.37±160.3	3270.44±121.35	2.66	0.08	2164.73±115.8	2458.04±94.88	1.03
		48	**0.01**	**0.01**	0.13	2926.11±83.16	2670.53±142.03	-1.25	0.5	2336.17±64.47	2459.92±164.76	0.78
		0	0.12									
	unknown	16	**0.01**	0.4	**0.01**	47.98±3.77	131.09±21.02	9.01	0.31	113.53±20.67	153.98±32.81	0.8
	LC-MS	24	0.07	0.6	0.29	77.24±7.23	97.58±15.07	1.41	0.48	95.91±12.23	77.09±22.51	-0.63
		48	0.67	0.16	**0.01**	90.48±7.56	53±9.91	-2.02	0.55	70.16±15.73	58.14±8.95	-0.31
		0	0.5									
	unknown	16	**<0.01**	0.89	**<0.01**	1164.15±54.63	232.88±101.09	-6.96	0.92	336.28±117.75	312.09±218.57	-0.08
	LC-MS	24	0.29	0.47	0.07	441.64±128.93	201.53±76.1	-0.93	0.4	679.85±228.31	414.33±195.04	-0.47
		48	0.27	0.69	0.12	456.34±178.25	144.33±35.92	-0.71	0.6	664±279.43	456.06±221.62	-0.3
		0	0.16									
	unknown	16	0.75	0.24	**<0.01**	5.66±0.58	11.51±0.74	4.08	0.2	5.94±0.59	8.34±1.77	1.65
	LC-MS	24	**0.03**	0.16	**0.01**	5.86±0.48	10.58±0.92	4.91	0.94	6.27±0.73	6.42±1.8	0.09
		48	0.48	0.42	0.21	7.48±0.66	6.49±0.35	-0.6	0.28	5.68±0.48	7±1.02	1.11
		0	0.1									
	unknown	16	0.17	0.81	0.06	848.57±12.24	1282.43±145.78	14.47	0.1	767.25±52.95	1097.23±189.18	2.54
	LC-MS	24	0.78	0.67	0.46	1073.08±92.52	1243.92±181.06	0.92	0.57	798.18±57.51	889.92±145.56	0.65
		48	0.27	0.16	**0.04**	908.67±83	1261.49±127.34	1.74	0.98	1039.93±159.54	1047.18±215.47	0.02
		0	0.63									
ureide	allantoin	16	0.16	0.61	**0.01**	54.95±37.81	396.19±104.49	38.62	0.24	155.63±66.62	311.43±112.23	0.95
	GC-MS	24	0.48	0.57	**0.05**	499.23±193.535	127.76±30.13	-0.96	0.09	328.88±135.6	74.07±13.79	-0.77
		48	0.26	**0.02**	0.36	478.97±86.71	612.4±117.41	0.63	0.11	195.57±52.25	367.63±79.49	1.34

LIST OF ABBREVIATIONS

MS- Mass Spectrometry

UPLC- Ultra Performance Liquid Chromatography

LC- Liquid Chromatography

GC- Gas Chromatography

ROS- Reactive Oxygen Species

MRM- Multiple Reaction Monitoring

WT- Wild Type