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Reporting Charcoal Rot in Chia and Developing a Susceptibility Assay

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Reporting Charcoal Rot Caused by *Macrophomina phaesolina* in *Saliva hispanica* and Developing an Assay Assessing Disease Susceptibility

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Introduction

About Chia

Chia (*Salvia hispanica*) is an herby plant native to Latin America grown for its use in food. Breeding of domestic chia varieties “Pinta” and “Tropic” to introduce the water tolerant calyx (seed containing structure) phenotype of Tropic chia into the commercially successful Pinta variety was conducted in the summer of 2018 (Fig 1).

Fig 1. Top: chia, ~180 cm. Bottom: rows of chia at field

Chia farmers did not face significant fungal damages likely due to the antimicrobial aromatics produced by the chia plant (Ref 1). And chia was only recently documented to be susceptible to Fusarium wilt (*Fusarium oxysporum*) (Ref 2, 3).

However our field trial of chia crossbreeds (F2 gen) experienced heavy decline and fatalities due to a fungus later identified as *Macrophomina phaseolina*. 70% of crossbreeds experienced damage (Fig 2) and around 50% of plants perished due to infection.

Fig 2. Damage to adult chia plants due to fungal infection weakening branches (top) and stems (bottom).

Definitive Identification of *M. phaseolina*

The novel identification procedure of *M. phaseolina* (Mahmoud & Budak, 2011) was used to definitively identify chia’s susceptibility to fungus (Ref 5). The characteristic microsclerotia (ball of dense hyphal structures containing nutrients for the fungi) were located on plate (Fig 3) and in the stems of plants inoculated with the fungus (Fig 4). Samples of stem tissue from inoculated plants produced identical fungal structures when plated on potato dextrose agar (PDA). Samples of the recultured fungus were genomically identical to field samples and *M. phaseolina* genome [NCBI full seq.] (Fig 5).

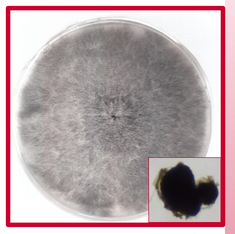


Fig 3. *M. phaseolina* on PDA & zoom of microsclerotia (x200).

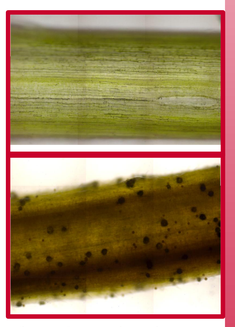


Fig 4. Comparison between non-infected (top) and infected (bottom) stem tissues for juvenile chia (x50 mag). Note microsclerotia.

Methods

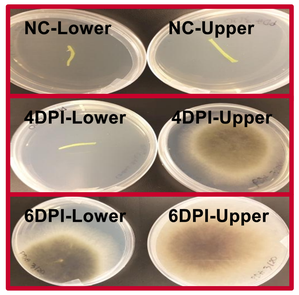


Fig 5. Stem tissue samples from chia plated on PDA to show infection progression at stem level.
Top NC no infect
Center: 4DPI Lo infect
Bottom: 6DPI Lo & Up infect

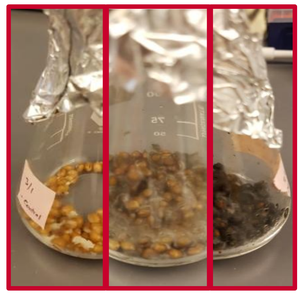


Fig 6. Stages of inoculum progression from 0 days post infection (left) to 4 DPI (center) to 8 DPI (right). Seeds blacken as the fungus envelops the seed producing dry, flakes of microsclerotia.

M. phaseolina Inoculum for Assay

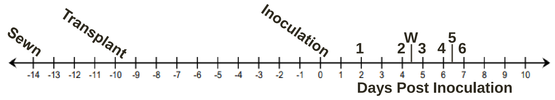
M. phaseolina is endemic to the equatorial range; and is known to infect over 500 plant species and produces varied pathology dependent on strain and host (Ref 4). To measure the specific pathology for chia, inoculum in the method of Bhandari (2017) was created to administer controlled amounts of fungus (Ref 7). On PDA *M. phaseolina* does not produce abundant reproductive spore structures (pycnidium) (Ref 6), thus a vector of inoculated wheat-seeds is used to achieve abundant sporing.

Wheat-seeds are soaked, drained, and autoclaved to sterilize and kill the seeds. Agar slants of *M. phaseolina* are crushed and added to the seeds to start the inoculum. The wheat-seeds spend 10 days in a dark incubator at 30±3 ℃ before the blackened seeds are added to chia plants (Fig 6).

Chia Grown in Sterile Conditions

Chia seeds are added to a sterilization solution of 5:3:2-EtOH-NaClO-H_2O, then thoroughly washed with water and plated on Murashige and Skoog (MSO) media. Seeds begin germination after 2-3 days and are transferred individually to Magenta GA-7 plastic boxes in 25 mL MSO media. They receive 24/7 light in a climate controlled growth chamber for 14 days of growth post sewing until they are inoculated by wheat-seed vector.

Boxes are opened in sterile laminar flow hood conditions to apply the inoculum. Single wheat-seed vector is placed in contact with the root to begin the assay. Plant pathology is closely monitored over the following week until the plant experiences fatality. Assay scheduled timecourse:



Results

Evaluation of Chia Pathology

Observation of chia inoculated with *M. phaseolina* showed consistent disease progression. Uptake of spores or hyphae into the root allows the fungus to produce microsclerotia in the plant’s vascular tissues. These microsclerotia are responsible for the browning of live tissue. Browning occurs in the root and progresses to the lower stem. Blocked vascular tissue causes wilting, and cotyledon damage as the infection progresses. Eventual true leaf damage and stem and leaf necrosis lead to fatality. Fatality occurs within 10 days, with few outliers (Fig 7).

Based on the repeatable pathology (n = 50) a scale was designed to measure disease progression and allow for graphing of symptoms over time:

0 = No symptoms
1 = Root browning
2 = Stem browning
3 = Cotyledon damage
4 = True leaf damage
5 = Major chloro/necrosis
6 = Plant fatality
W = Point of leaf wilt

Pathology Timecourse

Using the current inoculum procedure, chia experience fatality 7 days post inoculation ($p < 0.01$) (removed 2 outliers greater than 14 DPI). To account for variation in inoculum strength, the earliest sign of root browning was used as a normalizing measure:

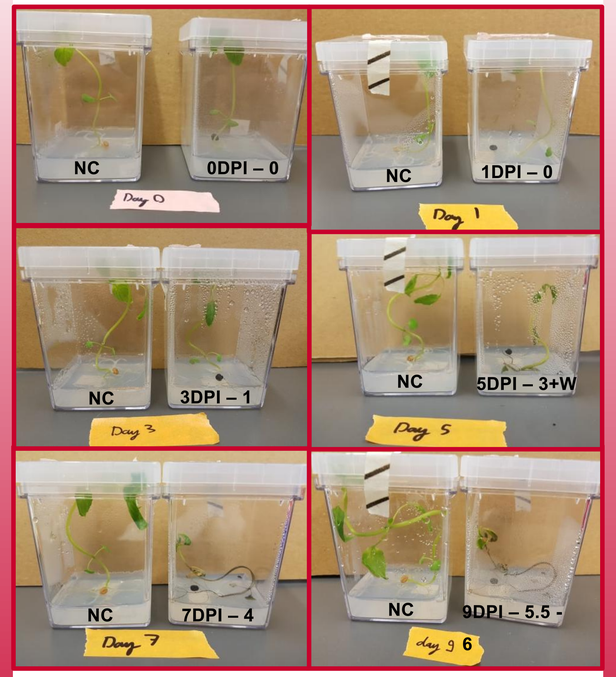


Fig 7. Examples for stages of disease progression up to 9 days post inoculation. Left are negative controls, Right is chia Pinta inoculated with *M. phas.* wheat-seed vector.

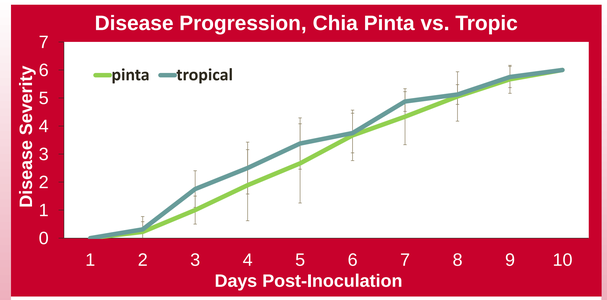
Ongoing & Forthcoming Projects

Assay for Susceptibility in Chia Tropic

The assay to evaluate susceptibility and potential disease resistance is being performed on the parental varieties of the crossbreed: Pinta and Tropic. Comparisons between Pinta and Tropic aim to identify if a more resistant phenotype exists in the domesticated cultivars. A preliminary test of disease progression has resulted in no significant difference in pathology between chia varieties (graph below). This insignificance may be due to the strength of the inoculant and the assay may need further refining before it can produce highly sensitive assessment of disease resistant phenotypes.

Refining the Assay

Moving forward, the biggest concern is that the inoculum is too potent and does not allow for an assay with the sensitivity to find tolerant phenotypes. The inoculum is magnitudes stronger than *M. phaseolina* found in soil and may be over saturating the plant with fungus. We intend to evaluate different ways to dilute the inoculum while retaining consistency in inoculum strength.



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