Project Title: Understanding the molecular mechanisms of resistance to INSV+Pythium wilt and tipburn in support of the USDA lettuce breeding program.

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Abstract

Monterey county harvests approximately 100,000 acres of lettuce (*Lactuca sativa* L.) valued at $1.2 billion per year. Local issues threatening this industry include, but are not limited to, the increased prevalence of Impatiens Necrotic Spot Virus (INSV) along with the co-occurrence of soil-borne pathogens such as Pythium wilt, and physiological disorders such as tipburn. INSV and the co-occurrence of Pythium wilt are relatively new problems in Monterey County since 2019, and considerable basic research needs to be done before solutions can be found. Resistance to tipburn is a continuing priority of the industry, but losses due to INSV and other diseases in research fields have prevented meaningful results from tipburn trials. Alternative greenhouse trials for tipburn resistant germplasm are necessary to continue to make progress in this area. Developing lettuce germplasm with genetic resistance to INSV and tipburn continues to be the most economical and environmentally safe method of control. Breeders are developing resistant lines, but continually need new information about the genetics and mechanisms by which the plant resists these threats in order to develop new, completely resistant varieties. Our goal is to support the USDA lettuce breeding program by developing an understanding of the physiological molecular mechanisms of tipburn and diseases, such as INSV and other soil-borne pathogens, and developing molecular markers for marker-assisted selection of stacked resistance to tipburn and disease.

Objectives

1. *To develop and optimize a greenhouse assay for screening breeding lines for tipburn. (In partnership with Dr. Kelley Richardson)*
2. *To screen existing breeding lines developed by Dr. Kelley Richardson using published markers (Macias Gonzalez et al. 2021) developed using a QTL study on cv. Emperor x El Dorado to confirm these markers are transferable to other genetic backgrounds. (In partnership with Dr. Kelley Richardson)*
3. *To develop and optimize an inoculation procedure for INSV and Pythium for observation of symptoms in the green house and growth chambers. (In partnership with Dr. Kelley Richardson and Dr. Frank Martin)*

Procedures:

Objective 1: We grew known tipburn-susceptible (cv. Green Towers) and tipburn-resistant (cv. Salinas) cultivars in greenhouses until 4 weeks of age, after which we intended to transfer them to a Conviron E7/2 growth chamber, under controlled humidity, temperature, and photoperiod conditions for 4 weeks. The growth chamber would have allowed testing of multiple temperatures and humidity settings at one time, in order to manipulate and test environmental conditions that have shown to induce tipburn in the scientific literature. However, the growth chamber was not available. We grew cv. Green Towers and Salinas in the greenhouse under relatively poorly controlled environmental conditions, but will continue this Objective when the E7/2 growth chamber becomes available.

Objective 2: We intended to harvest tissue samples from Objective 1 for DNA extraction and testing of molecular markers, however, because the growth chamber was unavailable and the greenhouse did not yield tipburn in tipburn susceptible plants, we did not test markers.

Objective 3: We took soil from a contaminated field at Spence Road, Salinas, following a variety trial that experienced significant Pythium infestation. We planted 4 cultivars of lettuce in a split-split-block design, in which some plants were grown on a heated map set to 70 degrees F, and other plants were left on the bench without temperature control. Plants were placed in trays that were given excess water to maintain 1-2 inches of standing water in the tray, or they were given sufficient water and drained to maintain wet but not saturated soil. The plants were grown for 6 weeks. Plants were then cut at the base, and total fresh weight biomass was recorded. Roots were visually inspected for advanced symptoms of Pythium.

Results and Discussion:

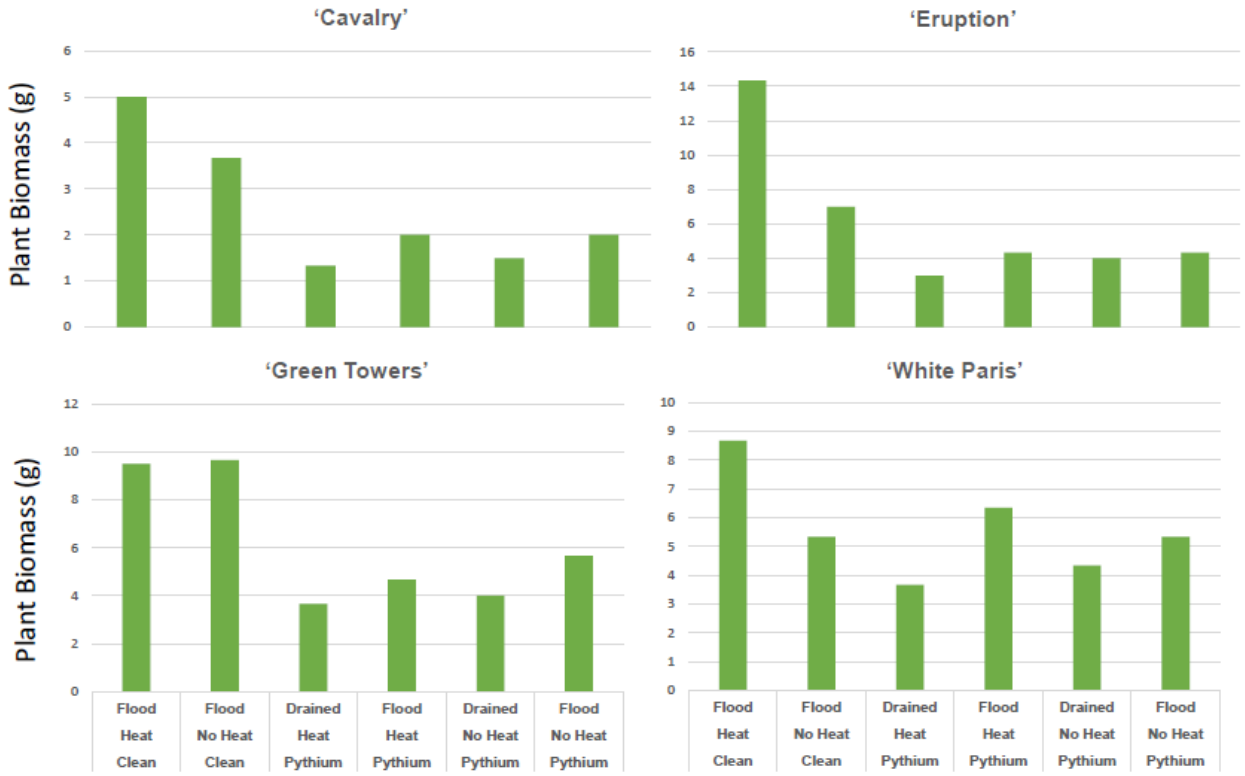
As we are a new lab with USDA-ARS, the intent of this proposal was to develop methods for screening for tipburn and inoculations that can be used in future experiments. We are in the process of purchasing equipment, hiring staff, moving to a new facility, and developing protocols that will be the foundation of all future research, and we are grateful to the CLGRB for their support of our efforts.

Due to worldwide shipping delays in 2021 and 2022, the E7/2 growth chamber ordered in August 2021 did not arrive until July 2022. Once it did arrive, a number of issues prevented timely installation, and since then, regular issues with the freon and condenser have led to multiple warranty-covered repairs, but no resolution. A second E7/2 was ordered in approximately August 2022, and is not expected to arrive until September 2023. We planted a small trial of tipburn susceptible Green Towers in the greenhouse, but were not able to induce tip burn. In an experiment intended to assay total polyphenol concentrations in 50 cultivars of lettuce in a growth room, we were able to induce tipburn in a small number of cultivars under low temperature and high humidity conditions. Plans for additional experiments are in progress to mimic those conditions in order to reproduce tipburn in those cultivars, and we are confident that additional experiments will be more productive.

As a result of the lack of codified method for inducing tipburn, we did not make progress on Objective 2. We believe we are making progress on Objective 1 now, and will continue to develop a reliable method for inducing tipburn in the growth chambers and greenhouse for future screening and for development of genetic markers.

Toward Objective 3, we did find significantly reduced plant biomass in Pythium-contaminated soil regardless of heat or water treatment compared to clean soil in all cultivars tested. It is not clear if the soil was contaminated with other pathogens to create conflicting results, however, it is likely that field conditions are also contaminated with other pathogens and create conflicting results. We did not observe advanced symptoms of Pythium in the roots.

Fig. 1: Average biomass of cultivars 'Cavalry,' 'Eruption,' 'Green Towers,' and 'White Paris' under treatment conditions.



Our staff worked closely with Dr. Richardson's group to learn the methods of manual inoculation with INSV, and we are confident that these methods are reproducible independently of Dr. Richardson's guidance. We are grateful for her attention and support, and will include these methods in future proposals. We will be working with Dr. Hasegawa closely to use his protocol for inoculating future experiments with viruliferous thrips. His staff are highly skilled in this protocol, and have graciously offered their support.

In closing, we are grateful for the CLGRB support and funding, and we will use the tools developing in this proposal as the foundation for future experiments to understand the molecular mechanisms of tipburn and plant-disease interactions in lettuce.