CALIFORNIA MELON RESEARCH BOARD GRANT REPORT 2019

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Characterizing and assessing risk of emerging fungal and bacterial pathogens of melons (and other cucurbit crops) across the nursery-field production continuum

Objective 1. Evaluate *Fusarium falciforme* as a potentially new crown rot pathogen of melon and other cucurbit crops in the California Central Valley, conduct cucurbit crown rot surveys, and use information to improve crown rot diagnostics

A. Proof of pathogenicity trials.

Results

We evaluated the pathogenicity of a *Fusarium falciforme* isolate recovered from crown rot of pumpkins in 2018. Although *F. falciforme* had not previously been reported as a pumpkin pathogen, this field was severely affected and *F. falciforme* was the only species consistently recovered, leading us to the hypothesis that it was the cause. We further hypothesized that other cucurbit species may be susceptible, including muskmelon and cucumber.

To test these hypotheses we set up a field experiment in a split-plot design with 3 replicate blocks per crop, with three crops, cantaloupe, pumpkin and cucumber, and at least five plants were inoculated per block. As controls we had 2 blocks of non-inoculated plants. Mature (~9 week old) plants were inoculated by wounding the crown with a 1 mm needle and placing a 1 cm diameter PDA-plug containing *F. falciforme* mycelia (one week old). Crown rot symptoms were evaluated seven weeks after inoculation. We found that the *F. falciforme* isolate was pathogenic to all crops, causing extended crown and stem lesions (Figure 1) in cantaloupes, pumpkins and cucumbers (92, 88 and 40 mm lesion length). There were no symptoms in the controls. To complete Koch's postulates and confirm that the isolate inoculated was recovered from the symptomatic plants, we conducted re-isolations and molecular indentation of the isolates recovered from infested plants. We confirmed that at least in 2 plants per crop inoculated *F. falciforme* was recovered . Currently we are conducting molecular identification of more isolates recovered.

This study supports the hypothesis that *F. falciforme* is a crown rot pathogen of cucurbits. Cantaloupe and pumpkin were the most susceptible hosts, with 2x longer lesions than cucumber. We have received reports of severe losses in commercial pumpkin fields in both 2018 and 2019 but not muskmelon. As it stands, *F. falciforme* has yet to be documented causing losses in commercial muskmelon fields. In tomato, this pathogen is very common but is commonly misdiagnosed as Fusarium wilt or other causes. *F. falciforme* is reported in several counties where muskmelons are grown (eg. Fresno, Merced, San Joaquin, Yolo, Sutter), so it seems likely that this pathogen is causing unrecognized losses in muskmelon; increasing awareness of this disease through outreach efforts can help increase detection and assess impacts.

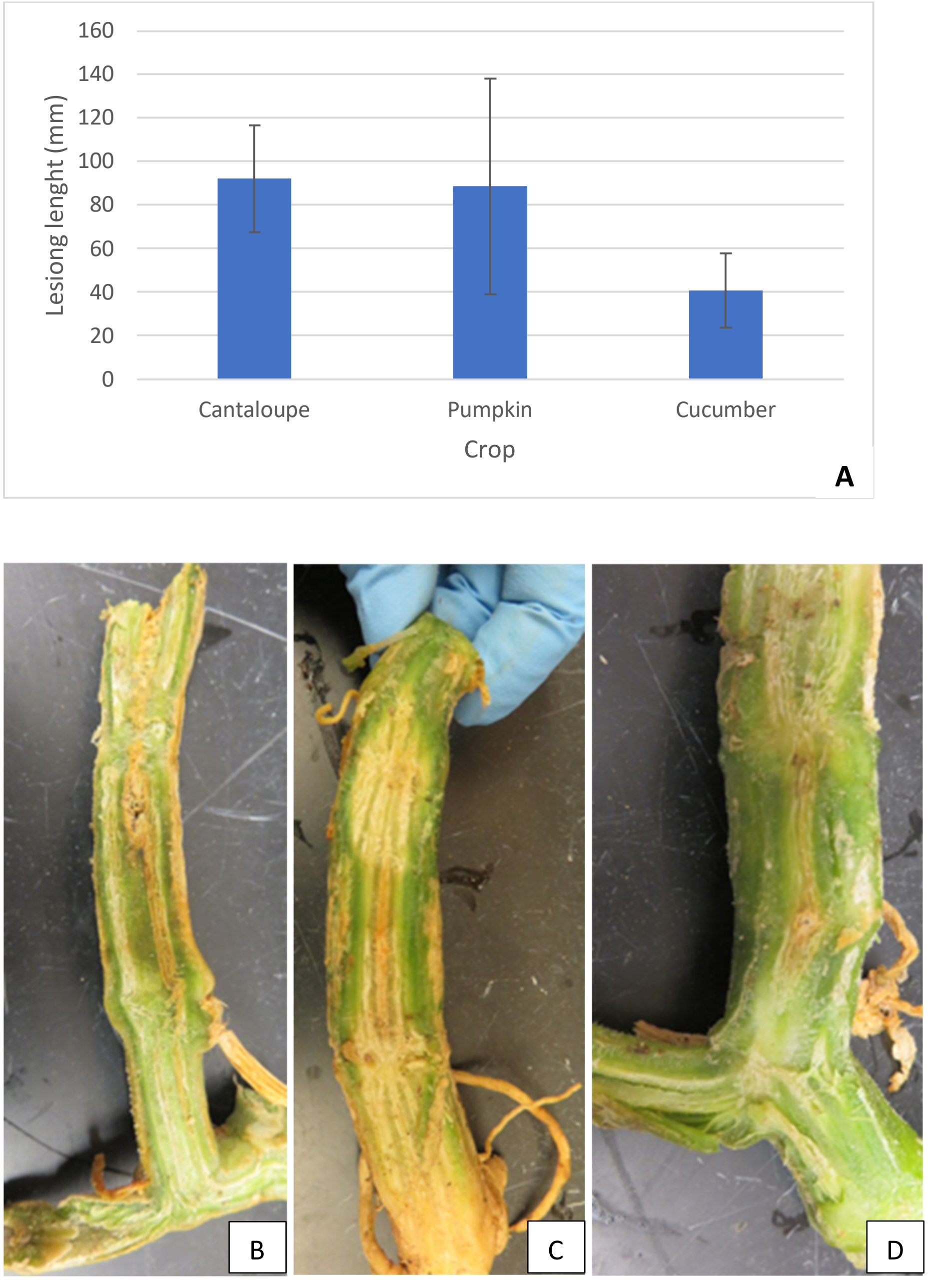

Figure 1. Pathogenicity of *F. falciforme* isolate recovered from pumpkins. **A.** Lesion length of crowns and stems of inoculated **B**. Cantaloupe, **C**. Pumpkin and **D.** Cucumber.

Pitfalls

Whole plant collapse symptoms reported from field samples were not observed during the experiment, since by the time we were evaluating the plants, they had already started to naturally senesce we were unable to evaluate wilting or any other symptoms. In future studies, inoculating at transplant could give more time for these whole plant symptoms to develop.

B. Cucurbit crown rot surveys in the Central Valley.

In 2019 we analyzed 10 cucurbit samples. We recovered *F. falciforme* from pumpkin and Peruvian ground apple. Fusarium wilt, southern blight, and seedling diseases (damping off pathogens) were also common.

Location	Plant (var.)	Tissue type; disorder	Diagnosis
Fresno	Watermelon	Leaf, spot	Unknown disease, possibly abiotic
Merced	Watermelon	Root necrosis, vascular discoloration, stem lesions, chlorotic and necrotic leaves	Unknown disease, possibly abiotic
Colusa	Watermelon for seed	Diploid seedlings	Damping off caused by *Pythium* and *Rhizoctonia* spp. and Charcoal Rot caused by *Macrophomena phaseolina*
Kern	Watermelon	leaf spot	Tentative: Alternaria leaf blight
San Benito	Zucchini (Romanesco Costata)	Crown; wilt	Fusarium wilt caused by Fusarium oxysporum
Colusa	Watermelon seedlings	whole seedlings	Charcoal rot, Southern blight
Glenn	Watermelon (Jubilee)	Whole Plant	Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum*
Merced	Peruvian ground apple	Crowns, roots, stems	crown and root rot caused by Fusarium falciforme
Shasta	Pumpkin	Crowns and roots	Fusarium crown and foot rot caused by *F. falciforme*
Sonoma	Winter Squash	Fruits with stem ooze/foaming	Bacterial stem rot
San Joaquin	Spaghetti Squash	vine decline + fruit lesions	Fusarium Crown and Foot Rot of Squash caused by F. solani likely causing both stem and fruit rots

Pitfalls

We did not receive any muskmelon samples this year and it is unclear whether this is because there were no muskmelon diseases or that we did not have good coverage of these crops.

Benefits for the industry

In this study we confirmed that *F. falciforme* is a pathogen of melons, pumpkins and cucumbers and establish that muskmelon is a highly susceptible crop. This information is valuable to increasing awareness of diseases affecting muskmelon, as well as improving diagnosis of muskmelon diseases. We will continue to monitor for this disease via diagnostic lab activities in collaboration with farm advisors. Should monitoring efforts reveal this disease to be of significant economic importance, this study had lain the foundation to conduct further research to identify at-risk areas of the state and provide effective management strategies for *F. falciforme*, including crop rotation recommendations and tolerant cultivar options. By monitoring cucurbit crown rot pathogens in the state, growers benefit by having knowledge of spread, prevalence and emergence of these detrimental organisms for the melon industry.

Objective 2. Conduct a hazard assessment to identify critical control points for bacterial and fungal transplant pathogens in greenhouses

To get a better understanding of the diseases affecting cucurbit transplants and the sources of infection, we conducted hazard assessment of critical control points (HACCP) in two cucurbit (muskmelon and watermelon) transplant nurseries in 2019. HACCP analysis can serve as a model to systematically identify and minimize the risk of pathogen contamination during the seedling production process.

The critical control points that we identified were as follows:

1) Reuse of trays. All nurseries reutilize trays in every planting season, steam-sterilization is the method most commonly used to clean them. This method reduces microbial load, as bacterial CFUs are significantly reduced after treatment (Figure 2); but inadequate tray storage after sterilization can accumulate dust, increasing the likelihood that pathogens like *Thielaviopsis*, *Botrytis*, that spread through dust contaminate clean trays (Figure 2). In assessments of tray cleanliness, we also evaluated the number of trays that sill contained soil/root plugs following sterilization. At one house 1/13 -1 /20 trays still had plugs. In the second house 0/20 trays had plugs in assessments of two pallets. Microbial assessment of plugs revealed that plugs contained high microbe loads. Re use of trays was thus identified as a critical control point. Species critical aspects of tray use were: Semi-adequate steam sterilization, inadequate tray shaking to remove plugs and storage conditions.

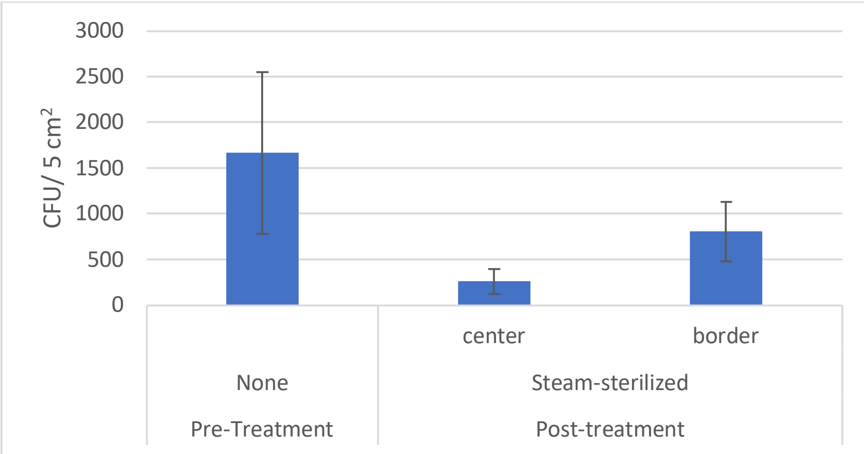

Figure 2. Colony forming units (CFU) per 5 cm^2 of bacteria recovered from swab sampling of reused trays before and after steam-sterilization stored at the center and at the border of storage pallets.

2) House sanitation practices, as they did not appear to be effective at reducing microbial load before and after sanitation treatment (Figure 2). Based on the fungal colony forming units (CFU) recovered per area sampled, CFUs were higher after treatment sanitation than before benches were treated. Inadequate bench sanitation methods was thus identified as a critical control point. In addition, workers did not have sanitizers in each house to clean footwear or gloves before entry; this could provide a critical control point, as it provides a way for pathogens to move between houses.

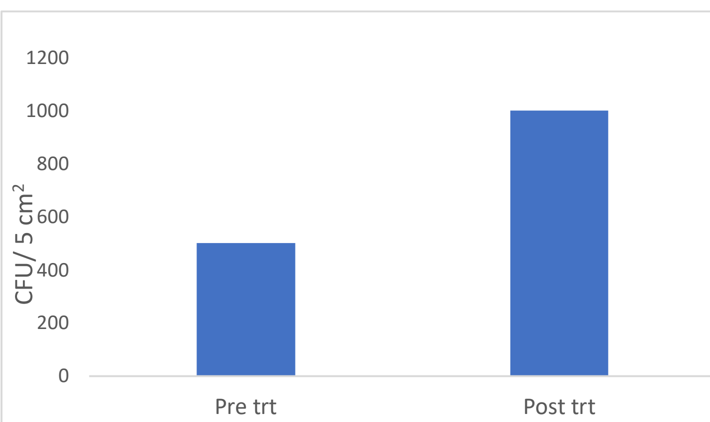

Figure 2. Fungal colony Forming Units (CFU) / 5 cm^2 recovered at pre and post bench sanitation with Oxyleaf.

3) Standing water to a close proximity from propagation trays, can increase the likelihood of water-splashed contamination with soil-borne pathogens (Figure 4). From standing water next to seedling trays, we recovered at least 300 and 500 fungal and bacterial CFU / ml of water respectively. Standing water may be a critical control point as it can provide a way for pathogens to splash into trays; in addition, the higher humidity associated with close proximity to standing water can reduce drainage, increasing root zone moisture and thus root rot risk.

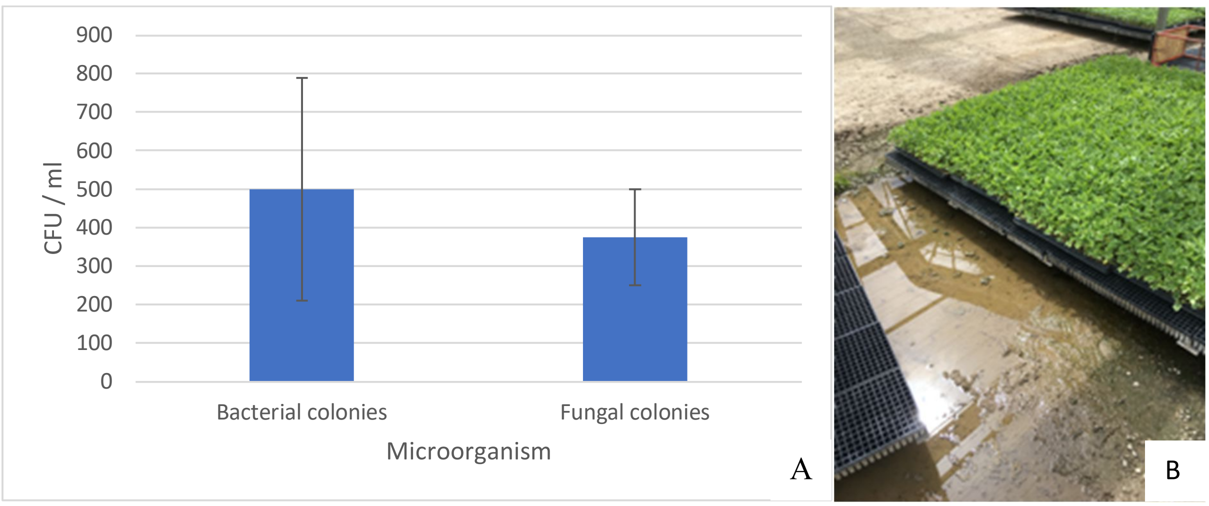

Figure 4. A. Bacterial and fungal colonies recovered from standing water, **B.** Standing water at the ground of a holding yard, at a close distance (15 cm) from the raised propagation benches.

4) Chemical management for black root rot. Historically, *Thielaviopsis basicola* causes severe black root rot resulting in significant losses in muskmelon transplant houses. Fungicide program assessments indicated that there are not specific chemicals in the fungicide regime which are known to have efficacy against the black root rot pathogen. Thus, another critical control point is introduction of fungicides with known black root rot efficacy into the management program.

Pitfalls

We conducted hazard analysis of critical control points (HACCP) in two greenhouses that produce melon-transplants. One of these houses did not grow melon-transplants this year due to a high incidence of Thielaviopsis in 2018, so were unable to monitor diseases; the second house only grew watermelon transplants. The first greenhouse is planning to grow melons again in 2020; we are requesting support to conduct a follow up HACCP in this nursery, to evaluate disease losses and further study pathogen sources. We also propose to conduct HACCP analysis at a third melon transplant nursery, which we lack resources to examine in 2019. We further propose work with all of the nurseries to evaluate methods to improve tray handling and bench sanitation, as well as modification of the fungicide program.

Benefits for the industry

This is the first systematic study to assess management targets for diseases in melon transplant houses. This work provides the foundation to investigate changes in cultural practices for melon producers that could increase plant quality going into the field, reducing transplant losses and increasing the viability of transplant use in the melon industry.