AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2018 – 2020

TITLE: Diagnosis and control of winter squash storage rots in the Willamette Valley, OR

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EXECUTIVE SUMMARY: Winter squash (*Cucurbita maxima*) grown for fresh market production in the Willamette Valley, OR has a profitable potential as a winter food, but is susceptible to fungal storage rots. In particular, a salmon-pink blossom-end rot affects well-known varieties immediately after harvest, causing fruit losses of 50-100% by Thanksgiving. This project aims to diagnose causes of squash storage rots and evaluate strategies to reduce their incidence.

Using culture methods, DNA sequencing, and artificial inoculations, a group of fungi that cause squash rot was identified. Importantly, three species of *Fusarium* -- *F. culmorum, F. solani,* and *F. incarnatum-equiseti* -- were strongly associated with diseased fruits, representing 72% of surveyed isolates.

We hypothesized that spores of *F. culmorum* or other *Fusarium* species are splashed onto flowers of squash plants by irrigation in summer where they cause a tiny, quiescent infection in the fruit that manifests into an active rot after harvest. In the field, flowers of squash were inoculated with *F. culmorum* and other *Fusarium* species. After harvest, stored fruit were evaluated weekly for fruit rot incidence. To date, *F. solani* has been associated with the greatest number of fruits (50%) rotting in storage and appears to be the most virulent pathogen tested. *F. culmorum* has been associated with rots of 10% of inoculated fruits.

We also hypothesized that disruption of irrigation-splashed pathogen spores onto flowers and developing fruit could be suppressed by either a physical mulch or a dryland production system. This hypothesis was tested by growing squash fruit to maturity on dryland, or irrigated and mulched plots. A plastic fabric mulch reduced disease incidence greater than straw mulch or control plots. Fabric mulch and control plots yielded similarly size fruits, while fruit size was reduced in straw mulch.

OBJECTIVES:

1) Demonstrate that fungal pathogens associated with diseased winter squash in the Willamette Valley reduce squash storability.
2) Evaluate the efficacy of reduced-irrigation production and organic mulches for the suppression of fruit storage rots of squash.

PROCEDURES:

Objective 1. Demonstrate that fungal pathogens associated with diseased winter squash in the Willamette Valley reduce squash storability.

Identification of potential winter squash rot pathogens. In fall 2017, fungi were cultured from rotted areas of squash fruit. Symptom were characterized by date rot started, color, location (floral-end, stem-end, side of fruit) and severity. Fungal morphology and DNA sequencing of barcoding regions, Internal Transcribed Spacer (ITS) and Elongation Factor 1α, were used to identify fungi. Four species of *Fusarium: F. culmorum, F. incarnatum-equiseti, F. oxysporum,* and *F. solani,* identified from fungal collections were grown on Spezieller Nahrstoffarmer agar (SNA) plates to produce inoculum. Healthy *C. maxima* squash were surface sterilized with 15% bleach and 11 fruits per isolate were inoculated. A sterilized one cm diameter cork borer was used to make a 0.5-cm deep wound in each winter squash. Plugs of each fungal isolate on SNA were cut with a 0.8-cm cork borer and placed in the wound, mycelium side down, and sealed with petroleum jelly. Control fruits (11 total) were mock-inoculated with a plug of sterile SNA. After 2 wk., rot appearance, diameter and depth were recorded. Fungi were re-isolated and identified to confirm Koch's postulates.

Determine if F. culmorum and/or other important squash rotting fungi colonize flowers. In summer 2018, forty 2-plant plots (4 ft. plot) of rot-susceptible winter squash cv. 'Sunshine' were established in an irrigated field with fabric mulch to control for irrigation splash of naturally occurring rot pathogens. Female, fruit-bearing, squash flowers were sprayed with liquid suspensions (10^5 spores/liter) of *F. culmorum* (2 isolates)*, F. solani, F. incarnatum-equiseti,* and a broth-only control. Flowers were sprayed the day they opened and monitored throughout the season. At maturity, fruits were harvested, counted and weighed. Fruit from inoculated flowers was stored in a closed barn bay at >32F ambient humidity. As rot symptoms developed, fungi were re-isolated to confirm identify of inoculated pathogen.

Objective 2. Evaluate the efficacy of dryland production and organic mulches for the suppression of fruit storage rots.

*Mulching effects on storage rot incidence. A*n additional twelve 5-plant (10 ft.) plots in the irrigated field of 'Sunshine' were mulched at planting with straw or fabric (6 of each). Six control plots did not receive any mulch. Plants were grown through the season and fruits were harvested and stored as described above. In November, December, and January, evaluations of the number of rotted fruits and the color and location of rot were recorded. Diseased fruits were removed from storage as they were detected.

SIGNIFICANT ACCOMPLISHMENTS TO DATE:

Identification of potential winter squash rot pathogens. 96 fungal isolates were cultured from 19 rotted *C. maxima* winter squash in 2017. On average, 12% of fruit surface area was rotted (severity ranged from 1% to 60% rotted surface area). *Fusarium* species were the most common fungi isolated from rotted fruit accounting for 72% of cultures (Table 1). They also were commonly associated with calyx-end rot symptoms. *Fusarium culmorum* and *F. solani* produced the most severe symptoms in inoculation assays. *F. incarnatum-equiseti* also

produced consistent lesions. *F. oxysporum* was the least aggressive among inoculated fungi. Mock-inoculated controls did not show any rot symptoms (Fig. 1).

Table 1. 2017 survey of fungi associated with rotting *C. maxima* winter squash fruits in storage between October and December.

Fungus[y]	Percent Isolated[z]	Rot color associated with fungus
Fusarium culmorum	11.46	Pink-White Rot
Fusarium incarnatum-equiseti	22.92	White Rot
Fusarium oxysporum	13.54	Pink-White Rot
Fusarium solani	6.25	White-Gray Rot
Fusarium sp.	17.71	White Rot
Penicillium sp.	7.29	Green Rot
Other Fungi	20.83	Black, Yellow, or White Rot

[y]Fungi cultured from rotting squash tissue, isolated in pure culture and identified morphologically and molecularly using ITS and EF1 gene regions.
[z]Percentage of each species out of all identified fungi

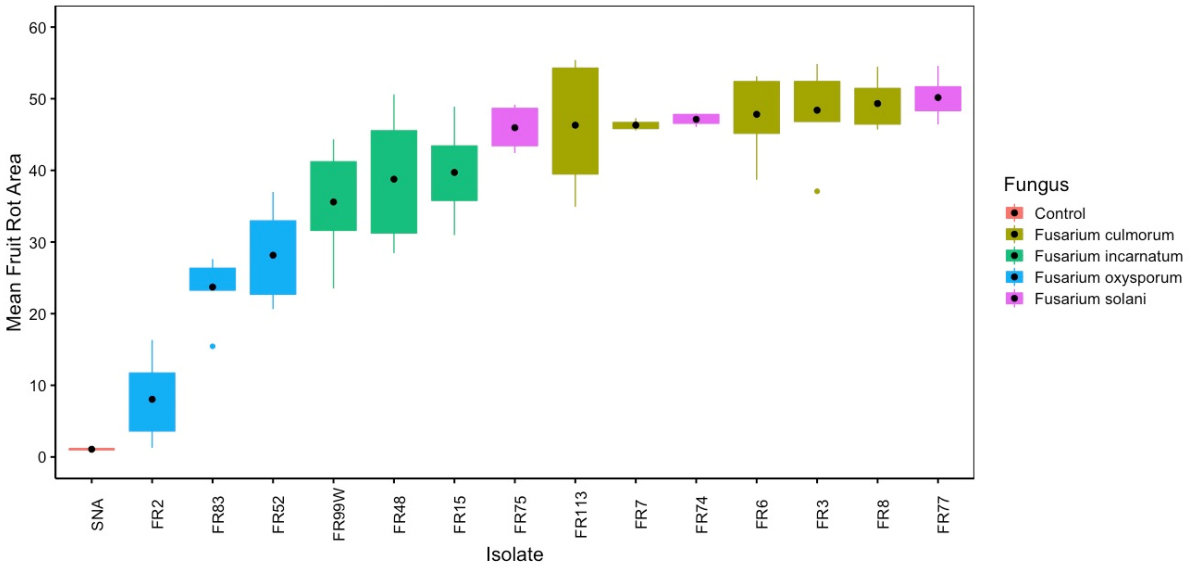

Figure 1. Mean surface area (cm^2) rotted of mock-inoculated *C. maxima* squash fruits at 2 weeks after inoculation with a fungal isolate. Colors depict different *Fusarium* species along with an agar-only control. Isolates were collected from surveys of rotted winter squash in 2017 growing season.

Determine if potential pathogens colonize flowers. For each of the five inoculation treatments, 55 flowers were inoculated (275 total). 59 fruits in total were harvested. In storage, rot symptoms began to appear on October 11th, and has continued through January. The treatments with the most rotted fruit are *F. solani* and the control (50% of harvested fruit; Table 2). Very few fruits have exhibited blossom-end, pink to white rot, though re-cultured fungi match the morphology of those inoculated. Only two fruits inoculated with *F. culmorum* isolates have shown rot symptoms.

Table 2. Counts of harvested fruits and rotting fruit from the field-grown, fungus-inoculated flowers of *C. maxima cv. 'Sunshine'* plants in 2018.

Inoculation Fungus	Fruit Harvested	Fruit Rotted[z]	Percent Rotted[z]
Control	12	6	50
F. culmorum 1	8	0	0
F. culmorum 2	12	2	17
F. solani	16	8	50
F. incarnatum-equiseti	11	1	9

[z]Count and percentage of fruit rotted as of January 25th, 2019.

Evaluate efficacy of dryland production to suppress storage rots. The field planting of dryland squash was unsuccessful due to extreme spring weather relative to the planting date of the experiment. Therefore, the efficacy of dryland production to reduce storage rot was unable to be evaluated.

Evaluate efficacy of organic mulches to suppress storage rots. The mulched field plots of 'Sunshine' squash yielded between 4 and 8 fruits per plot. Straw mulch plots had lower yields than fabric or control plots, likely due to seeds in the straw growing and becoming weeds and therefore competing with squash plants for nutritional resources (mean yield 3.15 kg per plot, $P \leq 0.05$; Table 3). Fruits in storage began to rot on October 11th and have continued through January. Control plots have the highest average percentage of rotted fruit per plot (59.4%) while fabric mulched plots have the lowest average percentage of rotted fruit (37.1%; Table 3). Four fruits showed the characteristic blossom-end rot and all of them were from control plots.

Table 3. Mean counts of harvested fruit and yield per plot in the 2018 irrigated mulch field trial of *C. maxima cv. 'Sunshine'* plants.

Treatment	Fruit Harvested	Fruit Rotted	Percent Rotted	Yield (kg)
Straw	4.2 ± 0.48[z]	2.2 ± 0.48	50.8 ± 7.57	3.2 ± 0.65
Fabric	5.7 ± 0.49	2.0 ± 0.37	37.1 ± 7.62	6.0 ± 0.65
Control	5.7 ± 0.33	3.3 ± 0.33	59.4 ± 6.27	5.5 ± 0.43

[z]Mean ± standard error.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:
Western SARE Graduate Student Grant in Sustainable Agriculture Project GW18-157, $25,000 from June 1, 2018 – March 31, 2019.

PUBLICATIONS

Rivedal, H.M., Stone, A.G., Johnson, K.B. 2018. First report of *Fusarium culmorum* causing fruit rot of winter squash (*Cucurbita maxima*) in Oregon. Plant Dis. 102:2659.

Rivedal, H. M., Stone, A. G., Severns, P. M., and **Johnson, K. B.** 2020. Characterization of the fungal community associated with root, crown, and vascular symptoms in an undiagnosed yield decline of winter squash. Phytobiomes doi.org/10.1094/PBIOMES-11-18-0056-R

FUTURE FUNDING POSSIBILITIES:
Western SARE Professional and Producer Grant in order to evaluate additional management strategies to reduce disease with on farm trials.