Investigation of *Aspergillus niger* causing Hull Rot and Conditions Conducive to Disease Development in Kern County

Project No.: **PATH18.Yaghmour**

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A. Summary

Hull rot is primarily caused by *Rhizopus stolonifer* and *Monilinia fructicola*. Infections by these fungi result in killing leaves, spurs, and parts of the shoot bearing the infected fruits. In Kern County, and the Southern San Joaquin Valley, *R. stolonifer* is more prevalent, and this fungus produces a toxin (fumaric acid) which moves from the infected fruit into the surrounding tissues killing the vascular tissues. Thus, hull rot affects future yields by killing fruiting spurs and wood. In the past years, orchards affected with hull rot in Kern County and other counties in the central valley showed the presence of *Aspergillus niger* growing among the hulls and shell in fruit with hull rot. Many samples were processed at Kearney Agricultural Research and Extension Center in Dr. Michailides' lab showed that hull rot samples from the San Joaquin and Sacramento Valleys were also infected with *A. niger* alone and/or *R. stolonifer*.

In 2020, we successfully reproduced the symptoms of hull rot in field by inoculating fruit with two different isolayes of *A. niger*. We also successfully reproduced the symptoms using two different concentrations of *A. niger* isolate used in 2018 and 2019 inoculations, this proves that the lower concentration 10,000 spores used in previous experiments were sufficient to reproduce hull rot symptoms and similar to using a higher concentration.

Populations of *A. niger* on fruit surface were also assessed again, and results showed that the highest population of *A. niger* was when almond fruits were at hull split stage c corresponding to hull being open less than 1 cm or 3/8" compared to earlier fruit developmental stages (unsplit, and b2). This is an important information and suggests that dust control may reduce the probability of population buildup on fruit surface and thus may play a role in disease management as a cultural way to manage this disease.

Commercial use of fungicides following grower's standards significantly reduced hull rot caused by *R. stolonifer* and *A. niger* by approximately 33-51% compared to untreated control. Furthermore, in a small-scale fungicide trial, almond fruit protected with a fungicide spray at stage c showed that fungicides in FRAC group 7/11 having the most disease protection against hull rot caused by *A. niger*. However, all protective fungicides significantly reduced hull rot when used to protect stage b2 (deep V).

B. Objectives

1. Complete pathogenicity tests with *Aspergillus niger* and study almond fruit susceptibility.

2. To assess disease incidence and monitor inoculum dispersal in the orchard.

3. Effect of tree water and nitrogen status on disease development.

4. Establish cultural and chemical control strategies of hull rot caused by *A. niger*.

C. Annual Results and Discussion

1. **Complete pathogenicity tests with *Aspergillus niger* and study almond fruit susceptibility.**

   Two different *A. niger* isolates, L-79 and L-30, were used to conduct the pathogenicity tests at the UC Kearney Research and Education Center in Parlier, CA. All inoculated almond fruit were at stage c at the time of inoculation. The two isolates significantly expressed hull rot symptoms compared to the non-inoculated controls ($P<0.05$, Figure 1A). Approximately 68.7 % of inoculated spurs with isolate L-79 expressed hull rot, while isolate L-30 expressed the disease in 47.6 % of inoculated spurs. In previous work, we have been using only isolate L-79. In pathogenicity tests, it is important to demonstrate that more than one isolate of the causal agent is reproducing disease symptoms consistently. This experiment shows clearly that *A. niger* will reproduce the disease symptoms. While L-79 reproduced more disease, the two isolates were not statistically different in reproducing hull rot symptoms expressing shriveled leaves and killing fruiting spurs.

   In another experiment, we tested two spore concentrations for their efficacy of reproducing hull rot symptoms. The purpose was to see if an increase in spore concentration may increase disease incidence or severity. Doubling the concentration of spores did not have any significant change in disease incidence (Figure 1B). This suggests that our use of 10,000 spores in our previous experiments was adequate to reproduce the symptoms of hull rot. It is worth noting that those experiments clearly demonstrate the ability of *A. niger* to reproduce hull rot symptoms, and any disease development in the control was most likely due to natural infection by *A. niger* spores in the orchard, which we

confirmed when we re-isolated *A. niger* from inoculated and non-inoculated control.

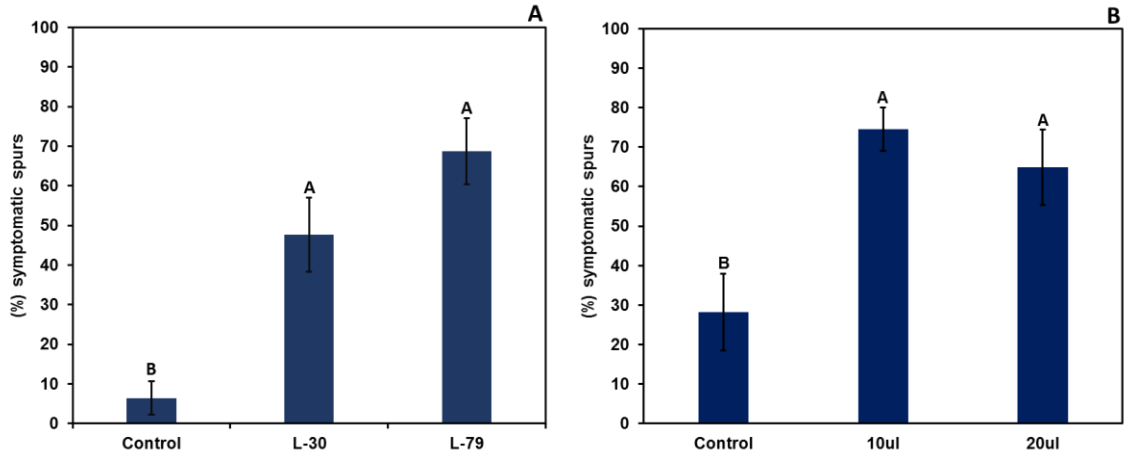

Figure 1. Percentage of Symptomatic spurs inoculated with spore suspension of two isolates of *Aspergillus niger* when fruits at stage c (split less than 1 cm) **(A)**, and at two concentrations of spore suspension of isolate L-79 **(B)**.

2. **To assess disease incidence and monitor inoculum dispersal in the orchard.**

In the past two years, we measured spore population on fruit surface over time by randomly collecting fruit, and we concluded that *A. niger* population increase as hull split progress with the highest population corresponding with fruit at stage c or later (fully split). In 2020, we measured spore population on fruit surface at the exact three developmental fruit stages when fruit are unsplit, at deep V (stage b2), and split less than 1 cm (stage c). The highest population of *A. niger* as well *R. stolonifer* peeked when fruit are at stage c (Figure 2), which confirms our conclusions and results from 2018, and 2019. The increase in *A. niger* population starts as the fruit starts to split (deep V stage), and the significant increase in *A. niger* population as the fruit is already split may be due to natural spore deposition from soil particles into the open fruit, and pathogens are actively growing at that point, and able to produce spores, which will result in increase in pathogen population. Also, it is important to note that stage c is the most susceptible stage, where previous experiments in 2018 and 2019 showed clearly that *A. niger* caused the highest disease incidence when almond fruit were inoculated at stage c. This information is helpful in disease management by looking at cultural aspects that will reduce the probability of spores reaching the canopy and be deposited on the hulls and inside the hull as they strart splitting. For instance dust reduction as hull start splitting may be of major importance, and certainly chemical control that will inhibit spore germination and protect almond fruits at stages b2 and c during hull split.

We also assessed the natural incidence of hull rot in two plots at the experimental orchard in Arvin, CA. The number of hull rot strikes is significantly higher in the northern plot compared to the southern plot (Figure 3A). Thus, we have trees with higher incidence in this orchard compared to other trees where the incidence is significantly less. This information will be used next year to test the effect of natural incidence on yield by understanding how the number of hull rot infection may affect yield. We plan to correlate the number of hull rot infection from each tree with the yield from same trees. We also measured again this year the natural incidence and infections of each hull rot pathogen in both experimental plots, and found that the percentage of symptomatic fruit infected with *A. niger* is significantly higher than fruit infected with *R. stolonifer* (Figure 3B).

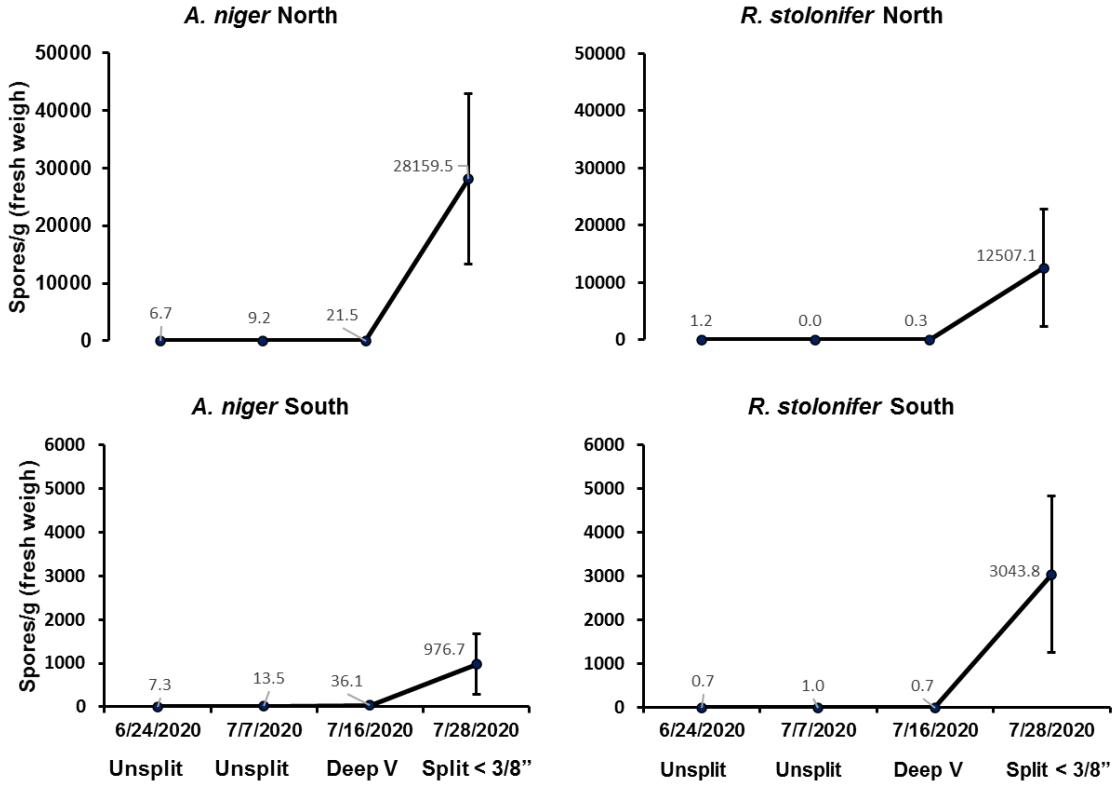

Figure 2. Population of *Aspergillus niger* and *Rhizopus stolonifer* on fruit surface in 2020.

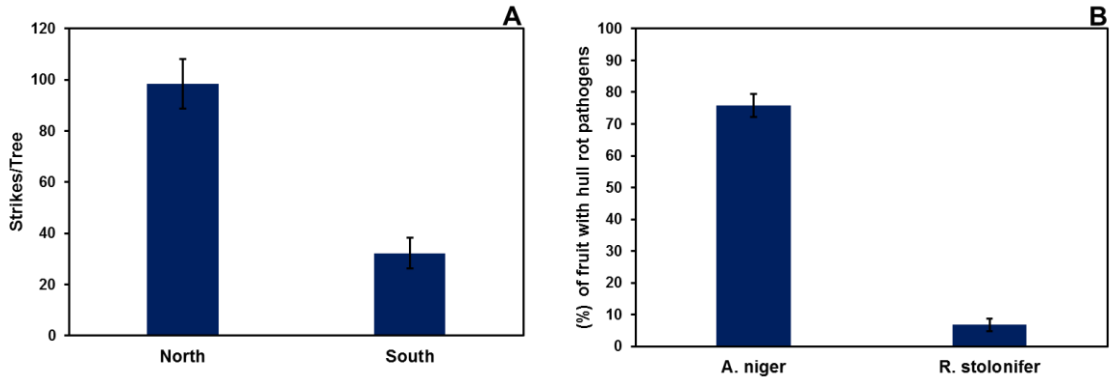

Figure 3. Natural incidence of hull rot in experimental plots in Kern County **(A)**, and percentage of fruit from symptomatic spurs colonized with *Aspergillus niger* or *Rhizopus stolonifer* **(B)**. Means represent the average five replicates (n=5)

3. **Effect of tree water and nitrogen status on disease development.**
   This objective was concluded last year and results from this objective were reported in last year's report.

4. **Establish cultural and chemical control strategies of hull rot caused by *A. niger*.**

   We repeated this year the fungicide efficacy experiments in a commercial orchard following grower's standards using four fungicides, Quash (FRAC 3) , Merivon (3/11), Luna sensation (7/11) and Abound (11) . Luna Sensation, Quash, and Merivon reduced the number of symptomatic spurs significantly by approximately 33-51% compared to control (Figure 4).

   In 2020, the experiment looking at the timing of fungicides application at two hull split stages (b2, and c) took place at the experimental plot in Arvin, CA. Fruits were sprayed with protective fungicides, using same materials mentioned above. The sprays were done with a hand sprayer 24 hrs before fruits were inoculated with *A. niger*. All fungicide treatments at stage b2 significantly reduced the number of symptomatic spurs between 43 and 55% (Figure 5A) compared to the untreated control. Furthermore, almond fruit protected with a fungicide spray at stage c showed that fungicides in FRAC group 7/11 had the most disease protection against hull rot caused by *A. niger* (Figure 5B).

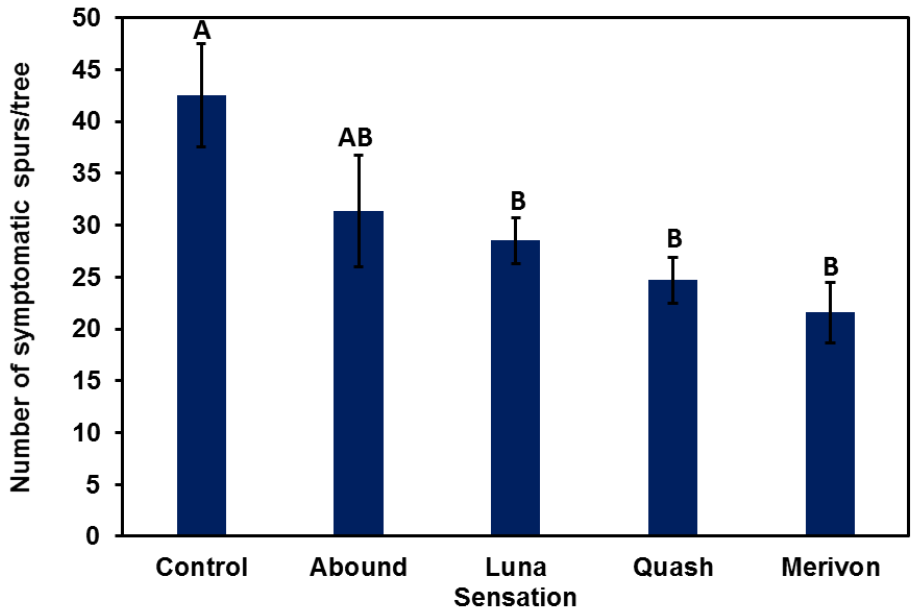

Figure 4. Effect of four fungicides on incidence of hull rot compared to untreated control in commercial orchard. Means represent the average of four trees in each block from four randomized complete block design (n=4).

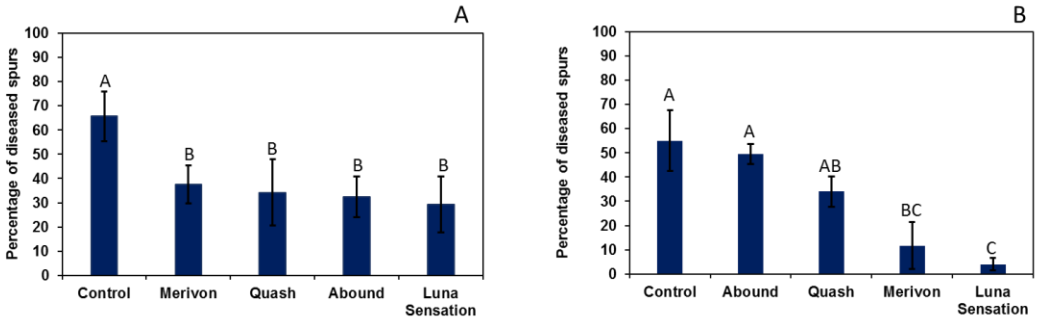

Figure 5. Effect of protective applications of three fungicides on incidence of hull rot compared to untreated control in inoculated branches with *Aspergillus niger* at (A) stage b2 (Deep V) and (B) stage c (Split < 1cm) (n=5).

D. Outreach Activities

1. Please describe outreach activities including the event description (date, location, topic of the presentation, aprox number of participants and type of audience)

Event	Date	Location	Topic	Number of attendees
Almond Conference	December, 2018	Sacramento	Investigation of *Aspergillus niger* Causing Hull Rot, and Conditions Conducive to Disease Development in Kern County	150

BASF Tree Nut Meeting	January, 2019	Bakersfield	IPM in Almond Disease Management	50
2019 Kern Almond Day	April 11, 2019	Bakersfield	Hull Rot management update	54
Wilbur Ellis Grower Meeting	April 16, 2019	Bakersfield	Almond Spring Disease and Hull Rot	8
Almond Conference	December 11, 2019	Sacramento	New and Expanding Plant Diseases: Hull Rot and *Ganoderma*	160
2019 Almond Workgroup Meeting	December 13, 2019	Davis, CA	Severe case of hull rot due to Neoscytalidium (presented by T.J. Michailides)	20
Helena grower's meeting	February 27, 2020	Bakersfield, CA	Kern County Disease Updates	70
CASP IPM meeting	May 20, 2020	Online	Hull Rot	113

E. Materials and Methods:

1. Complete pathogenicity tests with *Aspergillus niger* and study almond fruit susceptibility.

   Two experiments were conducted at UC KREC and in a commercial orchard in Arvin, CA. Fruits of the variety Nonpareil were inoculated when the hull was split at less than 1 cm (stage c) using spore suspension. In the first experiment, two isolates were used, L-79 and L-30. This experiment was conducted by placing 20 µl drop of 106 spore/ml suspension (20,000 spores). At the commercial orchard in Arvin, were inoculated as described earlier with either 10 or 20 µl drop of 106 spore/ml suspension of isolate L-79 to test if higher spore concentration can cause higher disease incidence. Re-isolations from disease fruit took place at the lab after surface sterilization of the fruit with 10% bleach for three minutes, and then rinsed with water two times. Fruit were then plotted dry on paper towels to dry before plating fruit tissue on acidified potato dextrose agar and incubated at 30 C for 72 hours.

2. To assess disease incidence and monitor inoculum dispersal in the orchard.

   Disease incidence between the two experimental plots in Arvin, CA was assessed by counting the number of symptomatic spurs. Ten spurs were also collected before harvest to assess the percentage of fruit infected with *A. niger*, *R. stolonifer*, or with mixed infections. Also, inoculum load in the same plots were

assessed by monitoring spore population on the fruit surface during the different fruit developmental hull split stages: unsplit, Deep V (stage b2), and split less than 1 cm (stage c). This was done by washing 10 almond fruits with sterile distilled water and plating the solution on plates with acidified potato dextrose agar (APDA)

3. Effect of tree water and nitrogen status on disease development.
   This objective was concluded last year.

4. Establish cultural and chemical control strategies of hull rot caused by *A. niger*.

   Previously, *A. niger* was screened for its *in vitro* sensitivity to various fungicides registered for use on almond. Representative fungicides that showed efficacy against *A. niger* were used in experiments in the field. Two experiments were setup, the first one was setup using Abound, Luna Sensation, Quash and Merivon applied with a commercial airblast sprayer combined with a NOW insecticide application when almond fruit in the orchard were at 5% hull split. The second experiment was setup to look at the timing of fungicides at two hull split stages (stages b2 and c). Fruits were hand sprayed with the same fungicides used above representing FRAC groups 3, 11, and 7+11 using spray bottles, and then inoculated with *A. niger* 24 hours later as described in Objective 1 with 20 µl drop of 10^6 spore/ml suspension (20,000 spores). All fungicide applications followed the label at the highest rate per acre.

F. Publications that emerged from this work

1. Mohammad Yaghmour, Brent Hotlz, and Themis Michailides. (2018) West Coast Nut Magazine. Issue: June pp 4-10.

2. Mohammad Yaghmour and Phoebe Gordon. (2019). Aspergillus Hull Rot in Almonds. Growing the Valley. P. Gordon. June 3.
   www.growingthevalleypodcast.com