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

Project No.: **PATH 18.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 ofleaves, 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 2018, we successfully reproduced the symptoms of hull rot in field by inoculating fruit with spore suspensions of *A. niger*. During the 2019 season, we repeated the pathogenicity tests in field inoculations of Nonpareil variety. A large number of fruits were inoculated with a spore suspension prepared from a sporulating culture of *A. niger*. While inoculated fruits reproduced the typical symptoms of hull rot with leaves shriveling and developing necrotic peduncles and tissue. Many fruit that were sprayed with water (uninoculated control) also developed hull rot, but it was mainly caused by *R. stolonifer.* Furthermore, some of the inoculated spurs had mixed infections (both *A. niger* and *R. stolonifer*). We will need to repeat this experiment again in 2020. We also repeated the experiments looking at the most susceptible developmental stage by inoculating fruit at the three developmental stages (unsplit, deep V (b2 stage), and split less than 1 cm (stage c) and found that Inoculated fruits at stage (c) had the highest percentage of spurs developing hull rot symptoms. In addition, populations of *A. niger*

on the surface of the fruit were assessed again, and results showed that the highest population of *A. niger* was observed later in July and through early August and corresponded with fruit that already split with less than 1 cm hull opening.

Nitrogen analyses of leaf samples collected in July (July nitrogen) were not significantly different between the two experimental plots, which showed differences in hull rot incidence for the second year in a row. The levels in nitrogen were within the optimal range and did not explain the difference in disease incidence in the two plots in this orchard.

In 2019, preliminary work looking at sensitivity of *A. niger* to different groups of fungicides was tested *in vitro* (lab tests) and in the field. *In vitro* tests showed that *A. niger* was sensitive to fungicides in FRAC groups 3, 7+11, and 7. An experiment using three fungicides belonging in the FRAC groups 3, 7+11, and 11, were conducted in a commercial orchard and all fungicides reduced the number of symptomatic spurs by approximately 39-54% as compared to non treated control fruit.

B. Objectives

1. To complete pathogenicity tests with *Aspergillus niger* and study almond fruit susceptibility: We will repeat the pathogenicity again in 2020; fruit susceptibility experiments determined the most susceptible stage which was when fruits were at stage c and the hull split was less than 1 cm; fruit susceptibility studies were completed and done for two seasons.

2. To assess disease incidence and monitor inoculum dispersal in the orchard. A spore dispersal study was completed in 2019. An increase of spore population on or inside the fruit corresponds to fruit at stage c during hull split.

3. Effect of tree water and nitrogen status on disease development. The July leaf nitrogen analyses did not explain the difference in disease incidence. However, disease incidence in the experimental site was significantly higher in trees with higher water stress during the hull split period.

4. Establish cultural and chemical control strategies of hull rot caused by *A. niger.* Perform *in vitro* (lab) screening of *A. niger* sensitivity to fungicides and field evaluation of chemical control at two hull split stages (b2 and c stages).

C. Annual Results and Discussion

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

   Disease symptoms were reproduced in inoculated fruits in 2018 with approximately 77% of inoculated spurs showing disease compared to 18% of spurs with fruit inoculated only with sterile water served as a control (Figure. 1A). The symptoms developed included shriveled leaves killing the spurs, and the peduncle showed necrotic tissue (Figure.1B). There was tissue discoloration that

was reddish in color. The discoloration developed in the tissue beyond the peduncle through the twig. This symptom is different from the black streaking developed from *Rhizopus stolonifer* infections. In 2019, the same experiment was repeated to complete Koch's postulates. However, *R. stolonifer* infected many fruits including the uninoculated controls resulting in more control fruits exhibiting hull rot symptoms and mixed infections with *A. niger* inoculated fruit (Figure. 1A). This part of this objective will be repeated in 2020.

Also, inoculations at three fruit developmental stages were conducted in 2018 and 2019. The results from both years clearly show that inoculated fruit when the hull split was less than 1 cm (Stage c) was the most susceptible with highest percentage of symptomatic spurs with inoculated fruit compared to non-split fruit, or fruit at the deep V stage (b2 stage) (Figure. 1C). However, there were approximately 40% of inoculated fruits at the deep V stage (b2 stage) that resulted in symptomatic spurs. This is important to take in consideration when looking at disease management. *A. niger* as well as *R. stolonifer* require wounds, and when fruit at the b2 stage start to split then a wound is formed. Fruits at stage C is already split allowing the spore deposition inside the inner surface of the hulls which provides a conducive environment for the fungus to grow and produce more spores. These results may play a role in chemical control decision to protect the most susceptible stage, however, 40% of fruits at stage b2 developed disease, suggesting that it is significant, and protecting this stage could be of value. For this reason, the results from this objective were used in a preliminary fungicide experiment looking at the effect of applying protective applications of fungicides at (b2) and (c) stage. Also this information provides justification for the impotance of reducing the dust during hull split. *A. niger* resides in the soil and spores can reach the canopy along with the dust clouds.

There have been also reports in 2019 regarding discoloration of the nuts due to the presence of the *A. niger* spores. However, I have not observed such symptoms in the field or from samples from processing plants. When it comes to field inoculations and evaluations of all symptomatic fruit causing hull rot in Kern County orchards, *A. niger* was observed growing only between the hull and the shell, and was not observed on the nuts (outer surface of hulls).

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

In 2018, disease incidence was assessed in the experimental orchard in Kern County and observed the disease in many other orchards during farm calls and visited an orchard in Fresno County which suffered from the disease in the previous season. Natural incidence was higher in the northern experimental plot compared to the southern plot which has a sandier soil (Figure. 2A). However, fruits collected from symptomatic spurs in the Southern plot had more fruits infected with *R. stolonifer* while fruits collected from the northern plot had more fruits infected with *A. niger* than fruits infected with *R. stolonifer* (Figure. 2B). In 2019, disease incidence in the Northern plot was significantly higher than the

Southern plot, similar to 2018 results (Figure. 2C). In 2019 fruits were not assessed for the percentage of the fruits infected with *A. niger*, *R. stolonifer*, or mixed infection.

Also, *A. niger* spore population on fruit surface was measured during July and early August and specifically during hull split. In 2018, the population in the northern plot was higher than the southern plot which may explain in part the higher percentage of natural incidence of the disease caused by *A. niger* in 2018. Populations increased significantly by the end of July. This corresponds with the susceptibility experiment where inoculated fruits that are split less than 1 cm had the highest disease incidence. This work was repeated in 2019 and we have seen the same trend of spore increase (Figure. 2D). This increase in the population when the fruits are at stage (c) could be due the ability of the fungus to grow inside the nut and produce spores. 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 inside the fruit such as dust management, or chemical control that will inhibit spore germination at stages b2 and c of the hull split.

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

   We measured the nitrogen status by looking at the July leaf analysis during 2018 and 2019. There was not significant difference between the northern and the southern plot in the July leaf nitrogen values (Figure. 3A). Although the leaf nitrogen content in 2019 was slightly higher or at the upper limit of optimimum July leaf nitrogen content (2.2-2.5%), this does not explain the difference in disease incidence between the southern and northern plots. It is well established from previous work that over fertilization with nitrogen plays a role in higher disease incidence of hull rot caused by *R. stolonifer*. However, to better understand the role of nitrogen overfertilization in hull rot biology, we need to understand the plant-fungal interaction at the hull split and if there are any factors that makes the hull more susceptible to fungal infection.

   Stem water potential was taken periodically during the growing season. Trees in the northern plot were more stressed during the hull split period compared to the southern plot (Figure 3B). However, more fruit in the northern plot were infected with *A. niger* than *R. stolonifer* in 2018. *A. niger* thrives in hot and dry conditions. It will be important for future work to address basic questions regarding how tree water status affects hull susceptibility in terms of what happens to the hull chemistry or water content that may affect fungal growth leading to disease development.

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

Aspergillus niger was screened *In vitro* against several fungicides. In 2019, preliminary work looking at sensitivity of *A. niger* to different groups of fungicides was tested *in-vitro* (lab) and in the field. *In vitro* tests at 10 ppm showed that *A. niger* was sensitive to fungicides in FRAC groups 3, 7+11, and 7. However, Abound fungicide (a.i azoxystrobin, FRAC 11) did not show any efficacy against *A. niger* (Figure 4A).

In an experiment, three fungicides, Quash, Merivon, and Abound, FRAC groups 3, 7+11, and 11, respectively, were sprayed on spurs bearing fruit in a commercial orchard. All fungicides reduced the number of symptomatic spurs by approximately 39-54% compared to control (Figure 4B).

In a preliminary experiment looking at the timing of fungicides at two hull split stages (b2, and c), fruits were sprayed with a protective fungicides, using same materials mentioned above. The application took place 24 hrs before fruits were inoculated with *A. niger*. Treatments at stage b2 showed the same trend as we observed in the commercial orchard and reduced the symptomatic spures between 20 and 65% (Figure. 4C). However, only Merivon and Aboound reduced the average number of symptomatic spurs when fruit were sprayed at stage c (Figure 4C). In both experiments we have noticed significant natural mixed infections by *R. stolonifer* in *A. niger*-inoculated fruit. We will repeat this experiment. However, we will focus more on commercial whole tree spraying instead of individual shoot spraying using handheld bottle sprayers.

D. Outreach Activities

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

E. Materials and Methods:

To complete pathogenicity tests of *Aspergillus niger* and study almond fruit susceptibility:

Nonpareil almond fruits were inoculated using spore suspension by placing a 10 µl drop of 10^6 spore/ml suspension (10,000 spores). To assess fruit susceptibility to *A. niger*, almond fruit at different developmental stages of hull split as described in the ANR publication Integrated Pest Management for Almonds were inoculated in the orchard using the same method. The stages that were inoculated included: Unsplit, Deep V stage of hull split (stage b2), and when hull split at less than 1 cm (stage c).

To assess disease incidence and monitor inoculum dispersal in the orchard:

In 2018 and 2019, disease incidence between the two experimental plots in Arvin were assessed by counting the number of symptomatic spurs. Only in 2018 that samples 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. This was done by washing 10 almond fruits with sterile distilled water and plating the solution on *Aspergillus* Differentiation Agar medium (also called AFPA) or acidified potato dextrose agar (APDA). Isolates were also collected from these petri plates.

Effect of tree water and nitrogen status on disease development:

Tree stem water potential was monitored weekly using a pressure chamber. Also, July nitrogen leaf analysis was determined by sampling leaves according to UC protocols and leaves were sent for analysis at a commercial lab. This data will be correlated with disease incidence collected from Objective 2.

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

Aspergillus niger was screened for its sensitivity to various fungicides registered for use on almond. The tests were done *in vitro* at 10 ppm concentration of each fungicide. Representative fungicides that showed efficacy against *A. niger* were tested in preliminary experiments in the field. Two experiments were setup, the first one was setup using Quash and Merivon applied with a commercial airblast sprayer combined with a NOW insecticide application on July 19th. Only Abound was applied 9 days earlier because of its PHI. The second experiment was a preliminary

experiment looking at the timing of fungicides at two hull split stages (stages b2 and c). Fruits were sprayed with 3 fungicides representing FRAC groups 3, 11, and 7+11 using spray bottles, and then inoculated with *A. niger* 24 hrs later as described in Objective 1.

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

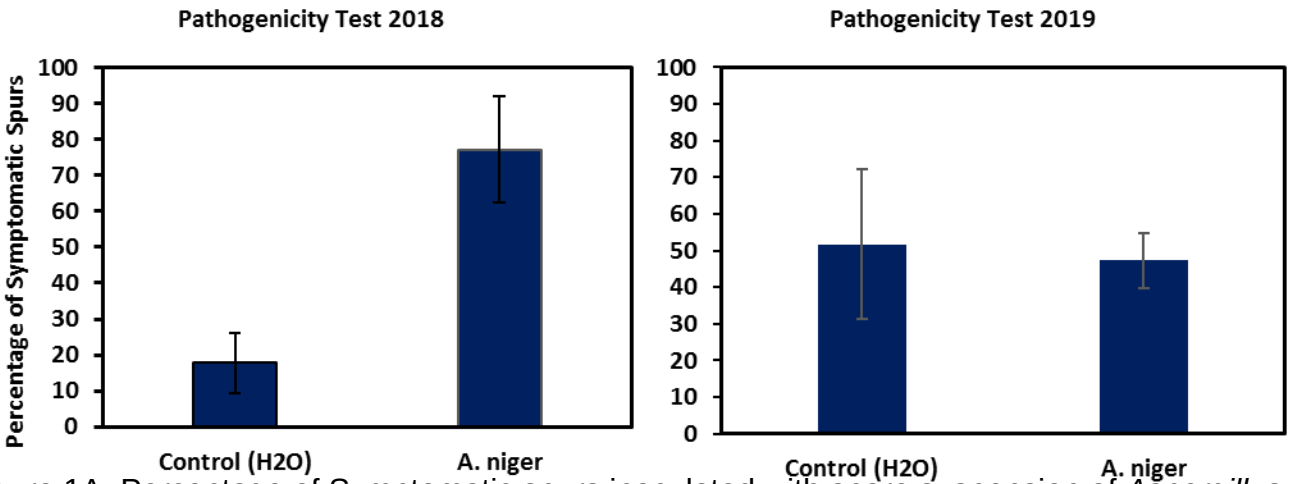

Figure 1A. Percentage of Symptomatic spurs inoculated with spore suspension of *Aspergillus niger* when fruits at stage c (split less than 1 cm) in 2018 and 2019.

Figure 1B. Symptoms of shriveled leaves and necrotic peduncle of inoculated fruit with *Aspergillus niger* (left), and percentage of symptomatic spurs inoculated with *Aspergillus niger* compared to control.

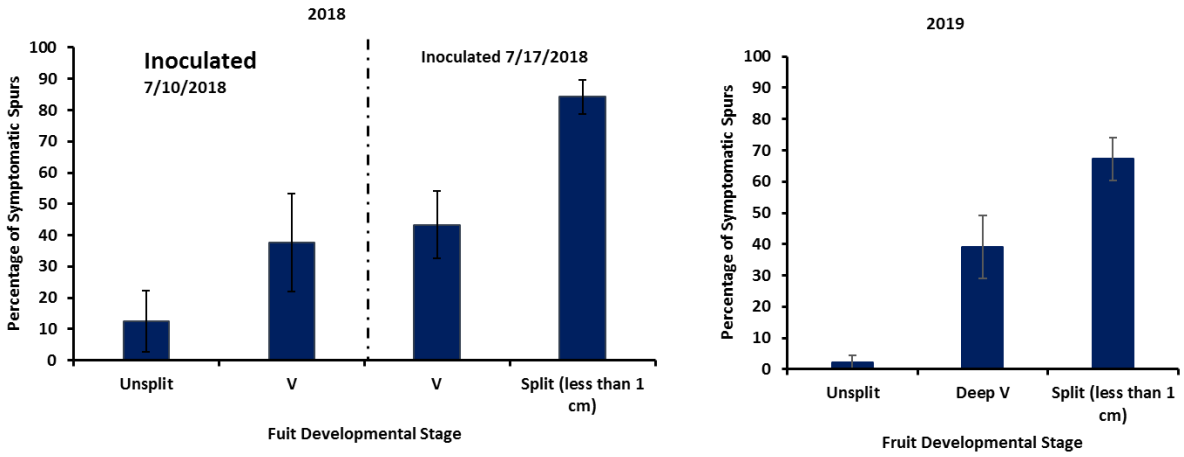

Figure 1C. Percentage of Symptomatic spurs inoculated with spore suspension of *Aspergillus niger* at different hull split stages in 2018 and 2019.

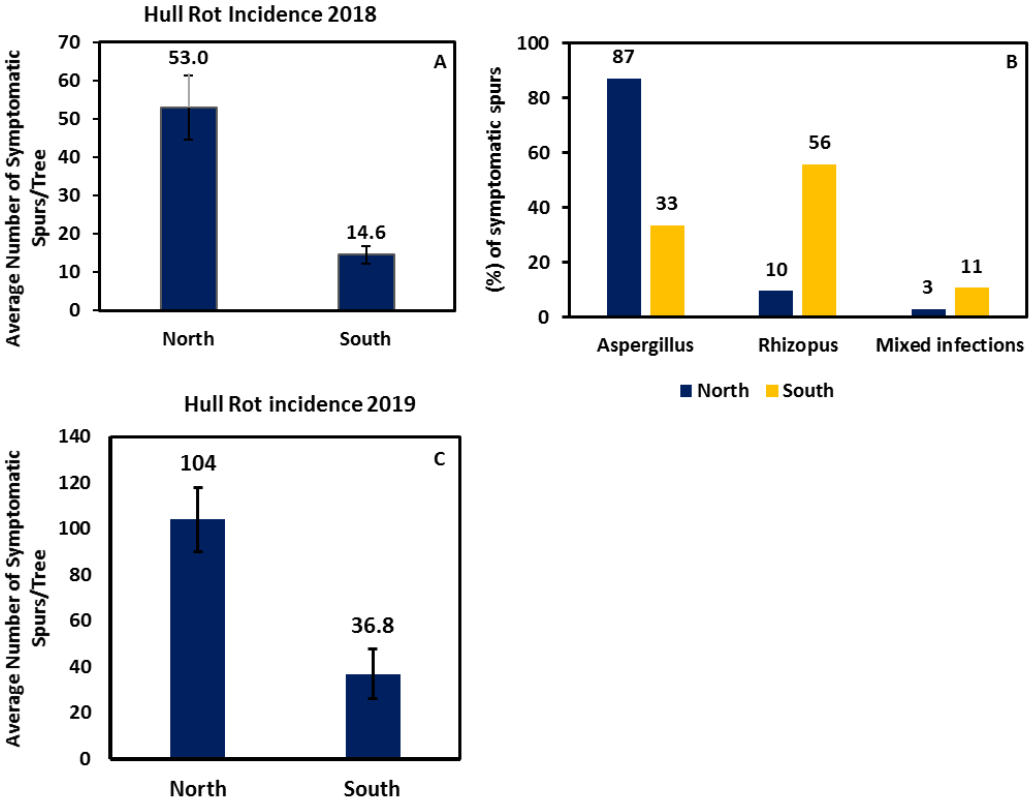

Figure 2. Natural incidence of hull rot in experimental plots in Kern County in 2018 and 2019 .

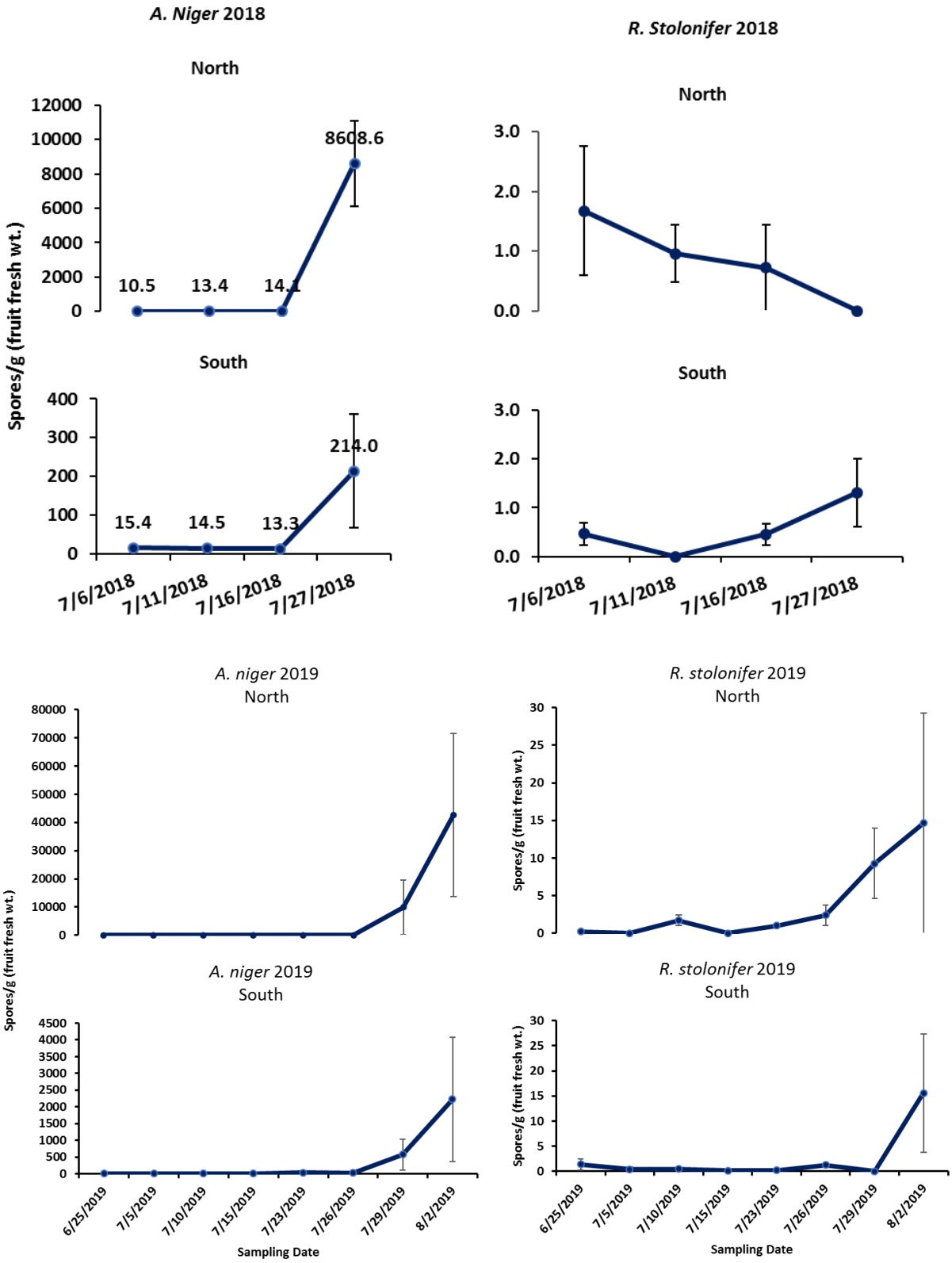

Figure 2D. Population of *Aspergillus niger* and *Rhizopus stolonifer* on fruit surface in 2018 and 2019.

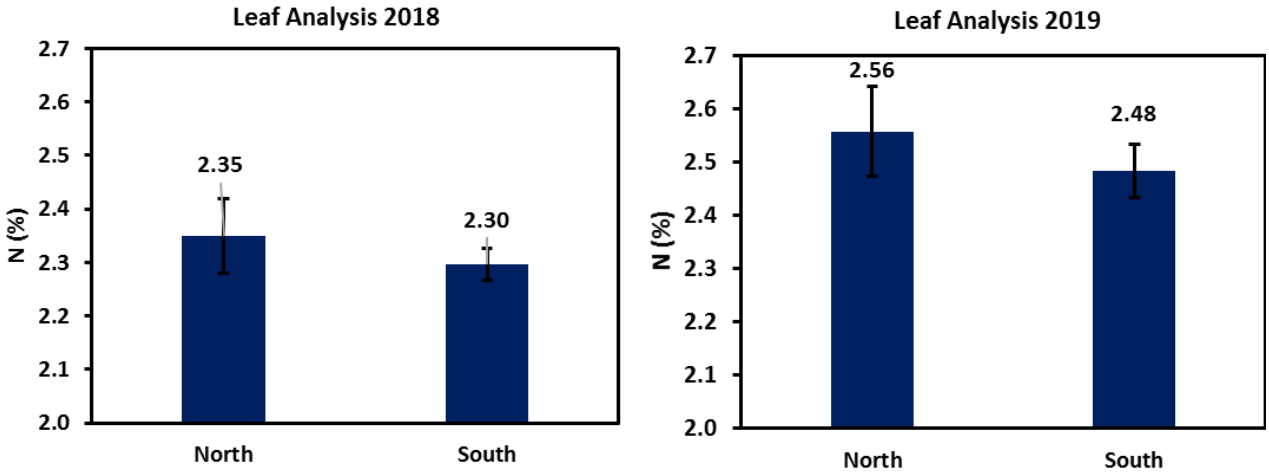

Figure 3A. July leaf nitrogen content in 2018 and 2019.

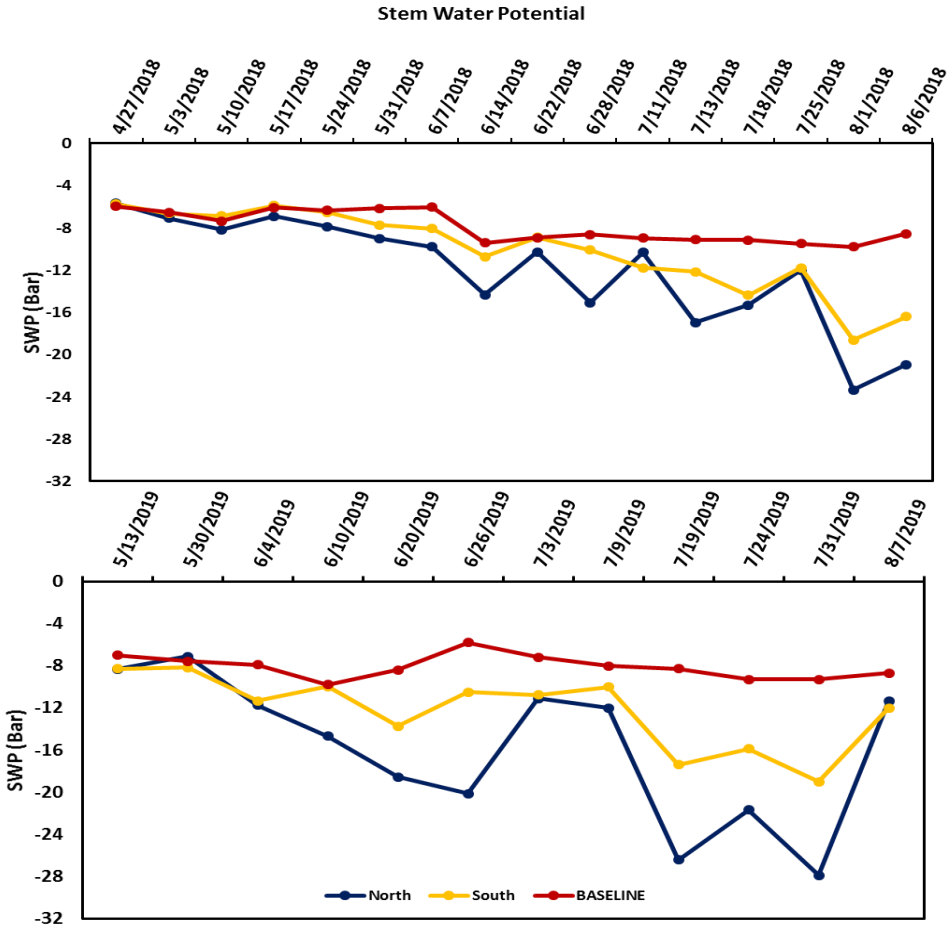

Figure 3B. Stem water potential for both experimental plots in Kern County in 2018 and 2019.

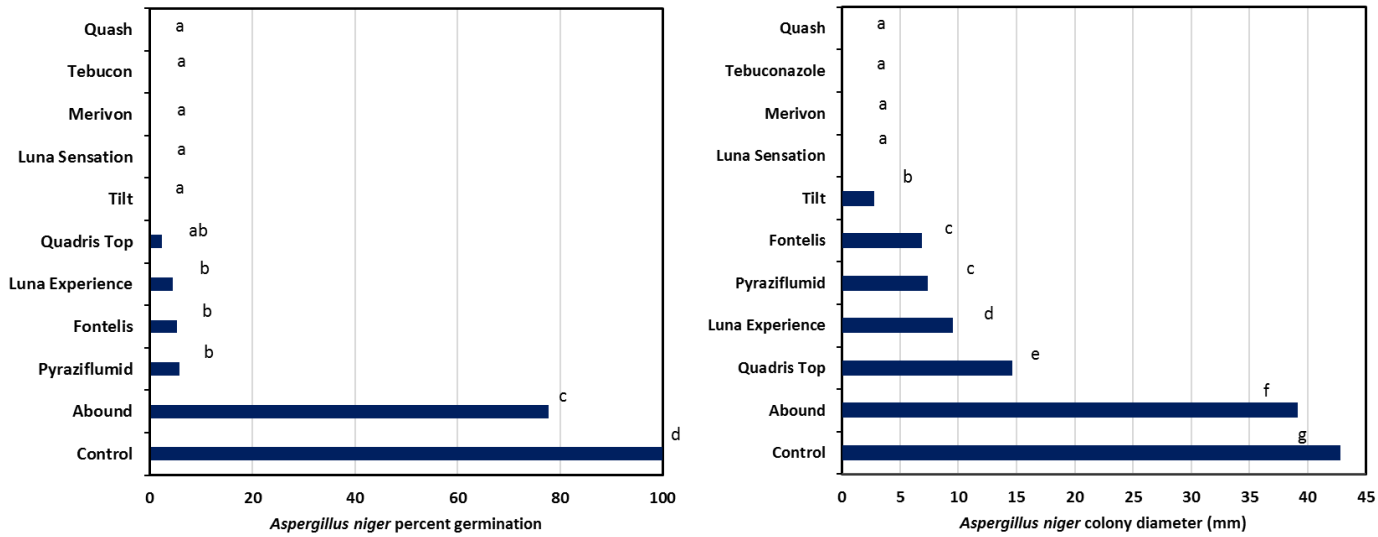

Figure 4A. *In vitro* sensitivity of *Aspergillus niger* to different fungicides at 10 ppm.

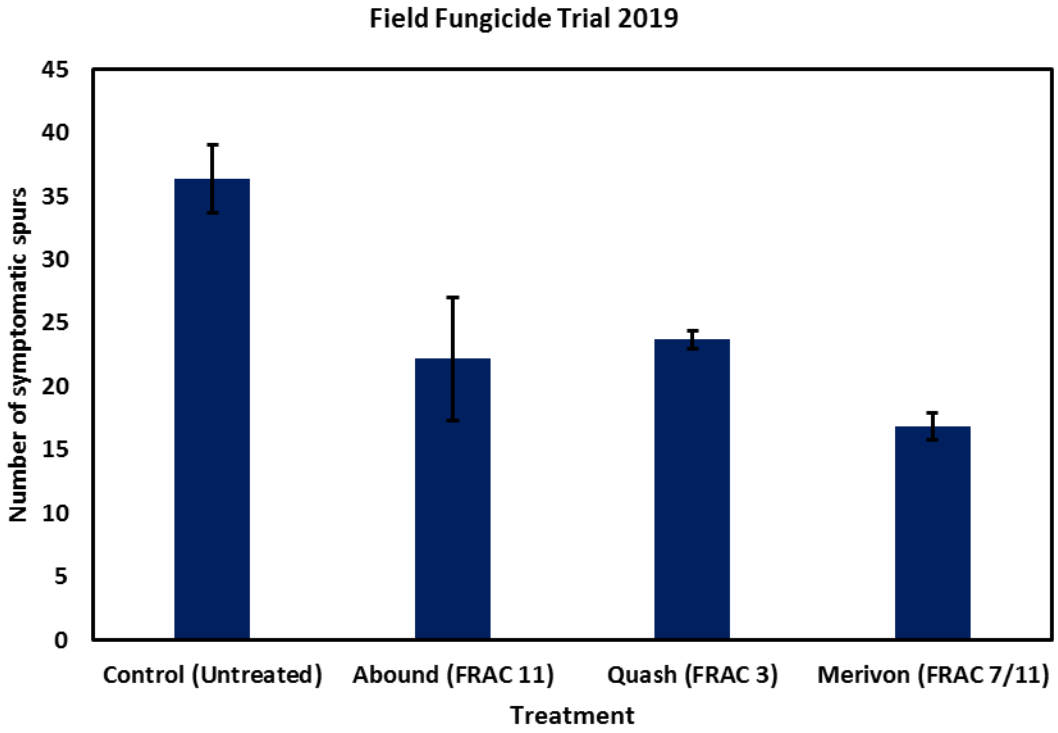

Figure 4B. Effect of three fungicides on incidence of hull rot compared to untreated control in commercial orchard.

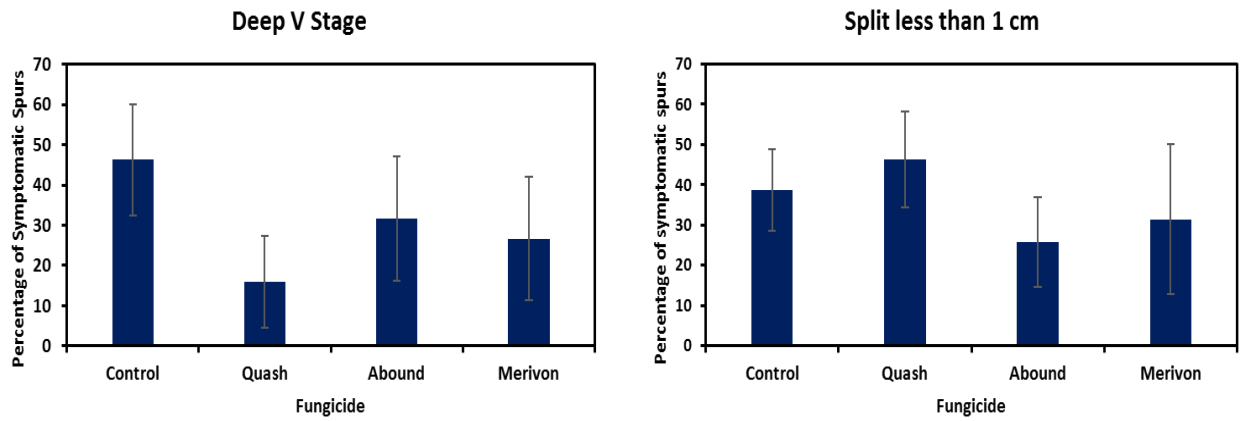

Figure 4C. Effect of protective applications of three fungicides on incidence of hull rot compared to untreated control in inoculated branches .