



# INVESTIGATING BOTRYOSPHERAERIA/PHOMOPSIS DISEASES OF WALNUT IN SAN JOAQUIN AND STANISLAUS COUNTIES, CALIFORNIA

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## Objectives

- 1** Investigate the frequency and distribution of Botryosphaeriaceae and Diaporthaceae fungi in walnut orchards from different locations in San Joaquin and Stanislaus counties.
- 2** Investigate when and under what environmental conditions spores of these fungi are released in three walnut orchards using spore-trapping study.
- 3** In vitro testing of three biocontrol products for the protection of walnut fruits.

## Background

In the last few years, Botryosphaeria and Phomopsis blight and canker diseases have been observed increasingly in walnut orchards in almost all walnut-growing regions in California. Main symptoms include cankers in branches and dieback of spurs resulting from infections moving from affected fruits (fruit blight) via the peduncle or shoots through leaf and peduncle scars. Results from previous research revealed the occurrence of ten different Botryosphaeriaceae species and two species of Diaporthe (synonym Phomopsis). Fungi in the Botryosphaeriaceae and Diaporthaceae species can be found throughout the year on diseased branches and may sometimes occur together on the same branch, which is difficult to diagnose in the field. These fungi overwinter on dead branches and shoots, and can develop both reproductive structures – pycnidia producing the water-splashed conidia and perithecia producing the airborne ascospores – with both spore types being dispersed during wet conditions.

Despite several management practices implemented to prevent major yield and economic losses caused by Bot/Phomopsis diseases, recent field survey results showed Diaporthaceae fungi to be the most prevalent fungal pathogen isolated from diseased walnut samples. Botryosphaeriaceae fungi were found occasionally. Although growers are making four to five fungicide applications per season to control this disease with inconsistent results, the persistence of Diaporthaceae spp. in walnut orchards has raised the question of whether these fungi have emerged as the main blight/canker/dieback disease of walnut.

## Results & Discussion

A spore-trapping study was undertaken to determine when and under what environmental conditions spores of these fungi are released. Based on colony counts, the population of Botryosphaeriaceae fungi was significantly lower than that of the Diaporthaceae fungi – which corresponds to the results of the field surveys that showed Diaporthe spp. to be the most prevalent fungal pathogens isolated from diseased walnut samples. Molecular identification for isolates recovered from diseased walnut samples and isolates recovered from spore-trapping studies revealed the presence of five new species of Diaporthe – three of them were found in both spore trapping and diseased tissues. The occurrence of these new species in walnut orchards represents new reports in California.

In the spore-trapping study, we analyzed the correlation between precipitation events, irrigation, and grinding of infected branches between tree rows (following maintenance/cleaning pruning) and Bot/Phomopsis spore release. Among these variables, we found a strong correlation between spore release and precipitation: as precipitation increased, spore release also increased, and spores were mainly captured from March to May, a period that coincided with late dormant season rainfalls. A new fungicide program was initiated this year to investigate whether an early spray timing would be effective to reduce the disease incidence.

We also detected high aerial dissemination of Phomopsis spores when grinding of infected branches deposited between tree rows in a mature orchard with a high incidence of Phomopsis disease; this information is of great importance as it helps to identify production practices responsible for the spread of these fungal pathogens within walnut orchards.

In orchards with sprinkler irrigation systems, low number of spores were captured during and following the first irrigation of the season. In addition, our results showed no correlation between further in-season irrigation events and the release of fungal spores of Diaporthaceae and Botryosphaeriaceae fungi. Here, we believe that the wetness/humidity in the orchard resulting from the first irrigation caused spores to ooze and be released from pycnidia in diseased tissues within the orchard.

In laboratory assays using walnut fruits, we tested the efficacy of three new biocontrol products – Julietta® (plant protection product based on living yeast), Epsilon  $\epsilon$ -Poly-lysine (EPL), and Vintec (Trichoderma atroviride SC1-based product) – against two fungal isolates of *Diaporthe ambigua* and *Neofusicoccum mediterraneum*. Preliminary results showed the biological yeast-based product Julietta® to perform well. We plan to repeat this experiment on a larger scale (laboratory assays) and evaluate the efficacy of these three biocontrol products in preventing fungal pathogen entry via pruning wounds. We are also assessing the pathogenicity of the new Diaporthe spp. recovered from walnut orchards.

