Spinach downy mildew epidemiology

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Downy mildew on spinach is caused by *Peronospora effusa* (also known as *Peronospora farinosa* f. sp. *spinaciae*). Downy mildew is the most widespread and destructive spinach disease in California. Though fungicide applications are available for the control of this disease in conventional production, adequate control measures are not available for organic production. Even with the availability of fungicides, the downy mildew inoculum is expected to increase or decrease under certain environmental conditions, knowledge of which can be helpful to time fungicide applications for disease management. A DNA-based assay was previously developed and validated to detect and quantify DNA levels of airborne *P. effusa*. Additional studies were conducted to determine if the pathogen is present in spinach leaf samples and seeds to curtail the introduction of such primary inoculum sources, if present.

Objective 1 of this research entailed further Analyze spinach seed for oospores that may transmit downy mildew. We have now examined 63 spinach seed lots by a seed wash-off method, in which 1000 seeds are shaken in water, the debris is centrifuged, and the sediment analyzed by microscopy. We have identified oospores in 7 of these lots, and identified sporangiophores characteristic of *Peronospora* sp. in another four seed lots. In two seed lots, numerous oospores were detected. To determine whether the oospores were viable in these two seed lots, two different viability tests were performed. The first test involved the use of a plasmolysis test. The plasmolysis test, the cell membrane shrinks to form a more compact sphere in the presence of a high salt concentration. In second test, the oospores were stained with a vital stain known as trypan blue. If the cell is viable, the membrane excludes the stain, and hence the cells are clear. We verified the presence of viable *Peronospora* oospores using both techniques for seed lots 4 and 12. Additional PCR analyses of DNA amplification confirmed that these seed lots contain *Peronospora* sp.

Objective 2 of this research entails quantification of airborne inoculum of *P. effusa* over two winter periods at a USDA spinach field plot, in 2013-2014 and 2014-2015 overwintering periods. Downy mildew infections were observed in the plot during both periods analyzed. An additional component of this objective entailed determining the level of airborne inoculum associated with the onset of disease development in the field plot. To accomplish this aim, a spinach plot of four beds and 150 feet in length was planted at the USDA station in Salinas on November 25, 2013 using the universally susceptible cultivar Viroflay. Spore trap impact samplers (spore traps) were placed on each of the four sides of the field. Rods were collected three days/week at all four traps. In the first detection period that spanned from November, 2013 to February, 2014, downy mildew disease was observed on the spinach in this plot beginning January 21, 2014 and was monitored until the plot was plowed under in mid February, 2014. DNA was extracted from the spore trap samples and *P. effusa*-specific quantitative PCR was performed from the spore trap samples obtained from the USDA station site from both the period 2013-

2014 and 2014-2015. The data analyses from the first period indicated that a peak of *P. effusa* detection occurred at the onset of disease occurrence. This peak could be easily distinguished from the very low background level of detection. Furthermore, as the wind during this period was predominantly from the northwest, the spore traps on the south and east sides of the field contained the highest levels of detectable DNA associated with the airborne inoculum of *P. effusa*. This work also further validates the use of the spore traps coupled with quantitative PCR to detect *P. effusa* in air samples.

Objectives 3 and 4 involved quantify airborne inoculum of *P. effusa* at four sites in the Salinas Valley and evaluation of the connection between spore trap data and the levels of downy mildew/weather data. Earlier results from two major pilot experiments, conducted in 2012 to determine the feasibility of airborne detection and quantification of *P. effusa* using the DNA assay and a spore trap system suggested that there is a "blanket" of airborne *P. effusa* spores/sporangia, blown in a southerly direction through the Salinas valley. Therefore, to further assess the amount of *P. effusa* present in the Salinas Valley over time, spore traps were placed at ~10-15 mile intervals in the Salinas Valley at four different sites in 2013 and 2014. Rods were collected three days/week at all four sites. Results suggest that there is an overall exponential increase in the amount of spores captured at each site over the course of the growing season. Despite this general increase in airborne inoculum, the spore load appears to be periodic, with clear but near-chaotic oscillations over the course of the season. Non-linear time series analysis shows that the dynamics of the inoculum are quasi-chaotic, suggesting that a mixture of deterministic and stochastic (i.e. random) factors impact spore load. The deterministic factors driving pathogen abundance are likely linked to crop area and infection rate. The stochastic factors, particularly weather, add noise to the system and make it difficult to accurately predict spore load. Factoring out the overall increase of spore numbers leads to the remaining short-term oscillations being even less predictable than when the increasing trend is present. Disease incidence ratings conducted in fields nearby spore traps in both 2013 and 2014 closely correlated with spore trap copy numbers. At all sites, an initial small amount of inoculum led to an increase in disease incidence, which was shortly followed by more airborne spores detected in the region. This cycle of inoculum-disease-inoculum may help us to determine when crops are most at risk to disease. We have gathered high quality weather data for 2013 and 2014 and will use these data to determine which weather variables are correlated with disease and spore load.

In summary, this work indicates that oospores of *P. effusa* are present in 9 % of the seed lots thus far analyzed using the wash-off method, of only 1000 seeds/lot. Moreover, we have confirmed the presence of viable oospores on two seed lots, using two different techniques. The transport and spread of *P. effusa* on seed is a concern since the oospore stage of the pathogen can survive 1-3 years, and the germination of these oospores can introduce *P. effusa* in new areas, and introduce new races of the pathogen as well. Oospores may also survive in the soil to initiate infections the following year. More work is necessary to determine if the viable oospores detected on spinach seed can infect spinach seedlings. We continue to monitor leaf samples to determine whether the pathogen is present in leaf samples from commercial fields.

The studies on the overwintering levels of airborne inoculum of *P. effusa* at the USDA field plot have revealed 1) presence of the pathogen even when not expected in a very dry winter when there were not other spinach plantings to our knowledge, and also 2) the level of airborne inoculum associated with the early onset of downy mildew in the Salinas Valley. Using this information, the placement of spore traps adjacent to fields can provide valuable information on the spore load that could be used to time fungicide applications locally. The analyses of the 2014-2015 overwintering data on the airborne

detection of *P. effusa* are ongoing.