

Biology and Epidemiology of Verticillium Wilt of Lettuce

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Objectives:

- Monitor Verticillium wilt and soil inoculum density in coastal California.
- Continue assessing seed lots and soil from seed production fields of private companies for potential *V. dahliae* infestation.
- Determine if airborne phase of the pathogen could be a threat in seed production fields.
- Simulation modeling of the impact of seedborne inoculum
- Determine pathogenicity of spinach isolates of *Verticillium dahliae* on lettuce and characterize to the race on lettuce.
- Continue the characterization of the spinach and lettuce pathogen populations.
- Continue the breeding program to identify and develop resistance in crisphead, leaf, and other lettuce types including screening of germplasm for resistance against race 2.

Verticillium dahliae causes severe losses in lettuce and other crops grown in coastal California. Since the mid-1990s, repeated outbreaks of Verticillium wilt have caused complete losses in many lettuce fields. Since the initial discovery of the disease on lettuce, 2-3 new fields on average had developed Verticillium wilt on lettuce. In 2008, the number of fields in which the disease occurred jumped to 13 and was equally distributed among the Salinas and Pajaro Valleys. The problem fields discovered until 2008 clustered around three hot spots with two of these located in the Salinas Valley and one in the Pajaro Valley. In 2009, however, the disease was discovered in 33 fields accounting for nearly a 50% increase over all the confirmed fields between 1995 and 2008. This number could very well climb even higher with the fall season still in progress. The three clusters around which the previous fields were located are progressively getting merged. There were several unique features among the new fields discovered this year. There were fields that had no history of spinach cultivation but also fields that have previously produced spinach. There were also several fields that had low incidence (<5%) of Verticillium wilt but the infected plants were randomly distributed within the field suggested inoculum source other than field equipment and personnel. Yet more fields had disease concentrated in a corner of the field that we have previously proven to have been initiated by contaminated equipment or personnel. Furthermore, there was at least one field which had only been fumigated with methyl bromide + chloropicrin and planted to strawberries last year. The disease albeit at low levels appeared on the first lettuce crop planted. On further enquiry, it was apparent that the field had been planted to a grass cover crop between strawberries and lettuce. There is a body of literature that has clearly established that although *V. dahliae* is a pathogen of dicots, it is able to colonize and reproduce on several grass species, onion and tulips that are monocots. These monocots can serve as symptomless carriers while also dramatically increasing the soil inoculum levels. The new fields were located in Watsonville and the entire length of the Salinas Valley. Of the 33 fields, at least five fields were abandoned because the loss was near-total. Incidence of wilt in other fields varied between 5-60%.

We continued the assessment of commercial lettuce seed lots for *V. dahliae* infestation. This year, we specifically focused on commercial lettuce cultivars currently marketed for salad mixes. Similar to last year, we requested seed companies for information on lettuce types and the locations where the seed was produced. Of the 12 companies approached this year, 6 submitted 71 seed lots for evaluation but the soil samples were available from only one company. The soil assay is still in progress and no data are available. Of the 71 seed lots evaluated on the *V. dahliae* semi-selective medium, two were positive for *V. dahliae* with one seed out of the 200 plated yielding the fungus for a seed infestation of 0.5%. The submitted seed was produced in the US, Chile, China, and The Netherlands and the two infested seed lots this year were produced in the US. Fumigation of the infested fields from last year has significantly reduced the seed infestation this year in the US. Since the soil infestation levels were too low to trigger extensive Verticillium wilt development in the seed crop,

the seed infestation could possibly have come from the airborne spread of the pathogen. Experiments to evaluate this were indeed successful. In addition, an even more complicated picture of pathogen transmission was discovered this year the pollen from one spinach germplasm line yielding *V. dahliae*. These results are preliminary and studies would be continued to confirm the role played by pollen in pathogen transmission into seed. In addition to *V. dahliae*, other fungi that produce conidiophores similar to *Verticillium* are occasionally observed on the seed. However, many of these do not belong to the genus *Verticillium*. Whereas the role these fungi play in the overall seed pathogen complex is unclear at this time, they do not in any way lessen the impact of *V. dahliae*.

Another major progress so far this year has been the development of a simulation model to determine how many infested seeds does it take to infest a field before *Verticillium* wilt occurs on lettuce, the duration over which *Verticillium* wilt epidemics develop given different seed infestation levels, the effects of disease incidence, microsclerotia added after each crop, tillage, prolonged intervals between crops, reduced production of microsclerotia on tolerant or resistant cultivars on subsequent *Verticillium* wilt development, and whether a tentative tolerance threshold can be established for commercial seed infestation. The development of this simulation model was only possible because data on the number of microsclerotia produced on infected lettuce plants and the inoculum density and disease incidence data were available from our previous work supported by the Board. For successful epidemics of *Verticillium* wilt to develop, a minimum of 3.5% of the planted seed is infested by the pathogen, this infested seed is planted consecutively over six to seven lettuce crops, all infected lettuce plants from these crops should produce a minimum of 3 million microsclerotia each, and at least 80% of these microsclerotia should survive between seasons. What role infested spinach seed planted in fields where lettuce crops follow is currently not known. After obtaining additional data, we will test various hypotheses in the simulation model over the coming year to answer this question.

Lettuce became a host of *V. dahliae* in the mid-1990s. The mechanisms involved in the expansion of the host range to encompass lettuce have remained elusive. Three hypotheses were deemed plausible, namely: a shift or adaptation in the local *V. dahliae* populations toward lettuce; or a sudden increase in population numbers in the region; or recurrent introductions of the pathogen into the area. These scenarios were primarily derived from the knowledge of the significant increase in the area planted with salad spinach in coastal California, and the published information on the high incidence of *V. dahliae* in spinach seed lots produced in multiple regions of the world. We developed and utilized a set of 22 unlinked simple sequence repeat (SSR or microsatellite) markers, which offered a set of robust co-dominant markers that may be used to retrace the evolutionary and migratory histories. Additionally, we generated 2603 AFLP markers (anonymous dominant markers), and utilized the sequences of the ITS and IGS rDNAs. Furthermore, strains were typed for their virulence on two lettuce differentials. Strains exhibiting race 1 and 2 virulence patterns were identified among spinach seed strains as well as strains from coastal California. Strains from spinach seed were successfully placed in each of the three clades obtained from the Bayesian phylogenetic analysis of the IGS rDNA sequences, indicating that spinach populations were not differentiated from populations collected in coastal California. From the SSR markers, no significant differentiation was measured among hosts in coastal California, which were also not significantly differentiated from spinach seed and Wisconsin ornamentals. Moreover, lettuce seed populations were not significantly differentiated. However, populations from tomato were significantly differentiated from the remaining populations. Migration analyses suggested that significant gene flow occurs among various geographic regions. Several strains from lettuce were assigned to spinach populations, and similarly many of the strains from spinach seed from various countries to varying sources. However, no strains were assigned to the lettuce seed population, even from coastal California lettuce crops. Lack of correlation between the virulence phenotype and genotype suggests that *V. dahliae* undergoes common recombination. Additionally, these results suggest that the high incidence of *V. dahliae* in spinach seed coupled with high planting density of this crop are likely sources of recurrent gene flow, and could be associated with the expansion of the host range of the pathogen into lettuce but direct evidence of spinach seed as a source of *Verticillium* wilt epidemics on lettuce is still lacking.

We have developed a PCR technique that selectively identifies the race 1 strain from *V. dahliae* isolates from a variety of hosts and has been validated with virulence assay on lettuce.