CALIFORNIA LETTUCE RESEARCH PROGRAM

April 1, 2013 - March 31, 2014

BIOLOGY AND EPIDEMIOLOGY OF VERTICILLIUM WILT OF LETTUCE

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SUMMARY

There were nine proposed objectives and the report includes the progress on six of them. Three objectives could not be completed because resistance to race 2 in the germplasm has not been confirmed. The six objectives being reported on are: a) continue pathogenicity evaluation of *V. klebahnii* and *V. isaacii* relative to *V. dahliae* on lettuce and other hosts; b) evaluate the effectiveness of *V. dahliae* race 1 resistance identified in lettuce against *V. klebahnii*, *V. isaacii* and *V. dahliae*; c) screen the ability of non-pathogenic *V. klebahnii* and *V. isaacii* to control wilt of lettuce caused by *V. dahliae*; d) to determine the potential of host-directed evolution of *V. dahliae* genotypes of differential virulence from a single genotype; e) to create a race 2-infested plot at the USDA Station; f) to continue the breeding program to identify and develop race 1 resistance in crisphead, leaf, and other lettuce types; and g) determine the effects of temperature on Verticillium wilt development in lettuce. Isolates of *V. isaacii*, *V. klebahnii*, and *V. dahliae* were evaluated for pathogenicity on lettuce and other hosts. *Verticillium dahliae* was pathogenic on most hosts, and a majority of isolates of *V. isaacii* and *V. klebahnii* was non-pathogenic. Some isolates each of *V. isaacii* and *V. klebahnii* caused extensive vascular discoloration in both lettuce and artichoke suggesting that species besides *V. dahliae* introduced into coastal California fields by spinach seed can also cause Verticillium wilt on lettuce and artichoke. The good news, however, is that the lines resistant to race 1 of *V. dahliae* also offer resistance against *V. isaacii* and *V. klebahnii*. Although detectable mutant forms of race 1 were identified, the threat of race 1 evolving over time into more virulent forms of *V. dahliae* appears to be low. Thus, unless new immigrants of race 2 strains are introduced into lettuce production fields, resistance deployed against race 1 could be quite durable. Despite our best attempts to create an infested field to screen for resistance to race 2, the microsclerotial densities in the field are not at a level where disease development occurs routinely. While our attempts continue to infest the soil by transplanting lettuce inoculated with the race 2 strain, we are also trying to determine the reasons for our past failure. Progress on the race 1 resistance breeding is in Ryan Hayes' report. Verticillium wilt developed well at temperatures between 20 and 30° C in growth chamber experiments, but the most extensive colonization of lettuce roots occurred at 20° C.

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PROJECT TITLE: **BIOLOGY AND EPIDEMIOLOGY OF VERTICILLIUM WILT OF LETTUCE**

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OBJECTIVES:

A. Continue the evaluation of the pathogenicity of *V. klebahnii* and *V. isaacii* relative to *V. dahliae* on lettuce and other hosts.
B. Evaluate the effectiveness of *V. dahliae* race 1 resistance identified in lettuce against *V. klebahnii*, *V. isaacii* and *V. dahliae*.
C. Determine the potential of host-directed evolution of *V. dahliae* genotypes of differential virulence from a single genotype.
D. Create a race 2-infested plot at the USDA Station.
E. Continue the breeding program to develop race 1 resistance in crisphead, leaf, and other lettuce types.
F. Determine the effects of temperature on Verticillium wilt development in lettuce.

PROCEDURES AND RESULTS:

Objective A. Determine the pathogenicity of *V. klebahnii* and *V. isaacii* relative to *V. dahliae* on lettuce and other hosts.

Methods. We hypothesized that *V. dahliae, V. isaacii* and *V. klebahnii* isolated from spinach plants may represent threats to other crops grown in California. To assess this hypothesis, four *V.*

isaacii (Cello-A-Tri-P, PD 341, PD 661 and PD 752), four *V. klebahnii* (PD347, PD407, PD458 and PD659) and two *V. dahliae* (Ls16 and Ls17) were tested on 4- to 5-wk-old seedlings of lettuce (Salinas and La Brillante), artichoke, cauliflower, eggplant, pepper, and tomato at the UCD greenhouse in Salinas and on spinach and strawberries at the UC Cooperative Extension greenhouse also in Salinas. All inoculated seedlings were transplanted into individual pots at both locations. Non-inoculated plants were maintained as controls. Ten seedlings per replication in three replications were inoculated and the pots were arranged in a complete randomized design on greenhouse benches. All plants were incubated for six weeks under a 13/11 light/dark regime in the greenhouse maintained at 20 $\pm$ 5 C except for spinach plants that were supplemented with 5 additional hours of light. After 8-10 weeks of incubation, each inoculated host plant was uprooted gently and soils were washed off the roots, and cut longitudinally to evaluate disease severity. Verticillium wilt disease severity was assessed as root discoloration on a 0-to-5 scale, where 0 = no vascular discoloration, 1 = 1 to 25% of the vascular tissue exhibiting discoloration; 2 = 26 to 50%, 3 = 51 to 75%, and 4 = 76 to 100% discoloration in the absence of foliar symptoms; and 5 = 100% discoloration and the presence of foliar symptoms typical of Verticillium wilt.

Results.

Pathogenicity on artichoke. *V. dahliae* isolates (Ls16 and Ls17) caused root discoloration. Plants were significantly stunted and leaves were dried in comparison to non-inoculated plants. Similarly, *V. isaacii* (Cello-A-Tri-P, PD 341, PD 661 and PD 752) and *V. klebahnii* (PD347, PD407, PD458 and PD659) also produced root discoloration. *V. isaacii* isolate 'Cello' produced significantly higher disease severities in comparison to *V. dahliae* isolate Ls16. However, overall, mean and median rankings of disease severity caused by isolates of *V. dahliae* were significantly higher ($P < 0.0001$) than those caused by *V. isaacii* and *V. klebahnii*.

Pathogenicity on cauliflower. *V. dahliae* isolates (Ls16 and Ls17), *V. isaacii* (Cello-A-Tri-P, PD 341, PD 661 and PD 752) and *V. klebahnii* (PD347, PD407, PD458 and PD659) caused no or negligible root discoloration in cauliflower. Plants looked healthy and were not significantly different from control plants. Median disease severity was highest for *V. dahliae* isolate Ls17 (0.57) and *V. isaacii* isolate PD341 (0.58), severity was the lowest (0.0) for *V. klebahnii* isolate PD407 and *V. isaacii* isolate PD661, respectively.

Pathogenicity on eggplant. *V. dahliae* isolate Ls17 exhibited severe root discoloration in most of the eggplants. Plants were stunted with dried leaves, in comparison to non-inoculated plants, However, *V. isaacii* (Cello-A-Tri-P, PD 341, PD 661 and PD 752) and *V. klebahnii* (PD347, PD407, PD458 and PD659) produced much less root discoloration. Overall, mean rankings of disease severity caused by isolates of *V. dahliae* were significantly higher ($P < 0.0001$) than those caused by *V. isaacii* and *V. klebahnii*.

Pathogenicity on pepper. All of the *V. dahliae*, *V. isaacii and V. klebahnii* isolates were unable to produce root and vascular system colonization or any vascular discoloration at the time of disease severity rating at plant maturity. Plants looked healthy and there were no significant differences between inoculated and non-inoculated plants.

Pathogenicity on tomato. On tomato cultivar 'Beef Steak', only the *V. dahliae* isolate Ls17 was able to cause some amount of vascular discoloration and wilting symptoms. *V. dahliae* isolates Ls16 and all of the *V. isaacii and V. klebahnii* isolates caused no Verticillium wilt symptoms. Plants looked healthy and there were non-significant differences between inoculated and non-inoculated plants.

Pathogenicity on spinach. *V. dahliae* isolates (Ls16 and Ls17) caused extensive root discoloration. However, none of the *V. isaacii* (Cello-A-Tri-P, PD 341, PD 661 and PD 752) and *V. klebahnii* (PD347, PD407, PD458 and PD659) showed any discoloration. Overall, median disease rankings of disease severity caused by isolates of *V. dahliae* were significantly greater ($P < 0.0001$) than those caused by *V. isaacii* and *V. klebahnii*.

Pathogenicity on strawberry. *V. dahliae* isolates (Ls16 and Ls17), *V. isaacii* (Cello-A-Tri-P, PD 341, PD 661 and PD 752) and *V. klebahnii* (PD347, PD407, PD458) caused clear root discoloration at the time of evaluating disease severity. However, one *V. klebahnii* isolate, PD659 caused lower disease severity compared to the other isolates. Overall, median disease rankings due to *V. dahliae* infection were significantly higher ($P < 0.0001$) than those produced by *V. isaacii* and *V. klebahnii*.

Objective B. **Evaluate the effectiveness of *V. dahliae* race 1 resistance identified in lettuce against *V. klebahnii, V. isaacii* and *V. dahliae*.**

Methods

The four *V. isaacii* (Cello-A-Tri-P, PD 341, PD 661 and PD 752), four *V. klebahnii* (PD347, PD407, PD458 and PD659) and two *V. dahliae* (Ls16 and Ls17) were tested on 4- to 5-wk-old seedlings of lettuce (Salinas and La Brillante), and race 1-resistant lines selected in consultation with Ryan Hayes included Annapolis, Eruption, Defender, Pavane, Little Gem, Sentry, Infantry, and Merlot. In addition, race 2 partially resistant PIs that were also evaluated included PIs 169511, 171674, 204707, and 226641. The experiment was conducted at the UCD greenhouse in Salinas. All inoculated seedlings were transplanted into individual pots. Non-inoculated plants were maintained as controls. Ten seedlings per replication in three replications were inoculated and the pots were arranged in a complete randomized design on greenhouse benches. All plants were incubated for six weeks under a 13/11 light/dark regime in the greenhouse maintained at 20 $\pm$ 5 C. After 8-10 weeks of incubation, each inoculated plant was uprooted gently and soils were washed off the roots, and cut longitudinally to evaluate disease severity. Verticillium wilt disease severity was assessed as root discoloration on a 0-to-5 scale, where 0 = no vascular discoloration, 1 = 1 to 25% of the vascular tissue exhibiting discoloration; 2 = 26 to 50%, 3 = 51 to 75%, and 4 = 76 to 100% discoloration in the absence of foliar symptoms; and 5 = 100% discoloration and the presence of foliar symptoms typical of Verticillium wilt.

Results.

V. dahliae isolates (Ls16 and Ls17) exhibited root discoloration when roots were cut longitudinally to evaluate disease severity on the race 1 susceptible cv. Salinas. Similarly, *V. isaacii* (Cello-A-Tri-P, PD 341, PD 661 and PD 752) and *V. klebahnii* (PD347, PD407, PD458 and PD659) also caused root discoloration. One of the *V. isaacii* isolates, Cello-A-Tri-P, caused

disease severity similar to *V. dahliae* isolate Ls16. Overall, median disease rankings of disease severity produced by isolates of *V. dahliae* were significantly greater ($P < 0.0001$) than those caused by *V. isaacii* and *V. klebahnii*, respectively.

In experiments using the race 1 resistant cv. La Brillante, *V. dahliae* race 2 isolate Ls17 caused extensive root discoloration and wilting symptoms. However, *V. dahliae* race 1 strain Ls16 and most other *V. isaacii* and *V. klebahnii* isolates caused little or no vascular discoloration and disease severity. In general, median disease rankings of disease severity produced by isolates of *V. dahliae* were significantly greater ($P < 0.0001$) than those caused by *V. isaacii* and *V. klebahnii*, respectively.

None of the *V. isaacii* isolates tested were able to produce root or vascular discoloration at the time of disease severity rating during plant maturity in any of the *V. dahliae* race 1-resistant and *V. dahliae* race-2 partially resistant lettuce cultivars. All of the lettuce plants looked healthy, and there were non-significant differences between inoculated and non-inoculated controlled plants. Thus, even if *V. isaacii* and *V. klebahnii* become serious pathogens of lettuce in due course, resistance already developed against race 1 of *V. dahliae* will provide resistance also on these two new pathogens.

Objective C. **Screen the ability of non-pathogenic *V. klebahnii* and *V. isaacii* to control wilt of lettuce caused by *V. dahliae*.**

Methods

V. isaacii and *V. klebahnii* are two recently described species of *Verticillium* that are common in agricultural soils in coastal California. When isolated and inoculated into economically important crops including lettuce and spinach, they induce much less severe disease symptoms compared to *V. dahliae*, and in many cases no symptoms at all. However, these weak pathogens are, in some cases, able to effectively colonize plant roots and vascular systems. Preliminary data suggest that colonization of plants with *V. isaacii* and *V. klebahnii* may actually be beneficial, because they may compete with and exclude the more aggressive *V. dahliae* from infecting plant tissue. We are currently studying the interactions of these species in lettuce and eggplant (model system) using the following experimental design:

Table 1. Experimental design to study the interactions of soilborne *Verticillium* species and their role in disease and potential disease suppression

	Experimental treatment		
Treatment	**1st Inoculation**	1 week	2 weeks
1	Ls16	.	.
2	PD660	.	.
3	PD659	.	.
4	Ls16	PD660	.
5	Ls16	.	PD660
6	Ls16	PD659	.
7	Ls16	.	PD659
8	PD660	Ls16	.
9	PD660	.	Ls16
10	PD659	Ls16	.

11	PD659	.	Ls16
12	Ls16 + PD660	.	.
13	Ls16 + PD659	.	.
14	Control		

Note: Ls16 = *V. dahliae* race 1; PD659 = *V. isaacii*; PD 660 = *V. klebahnii*

Experiments are being conducted at the UC Davis greenhouse in Salinas; the fourteen treatments are replicated three times, with three pots in each replicate and arranged in a complete random block design. The first round of experiments is on-going at this point and disease severity will be evaluated within 4 weeks.

In addition to these sequential inoculations and co-inoculations, we will also investigate whether *V. isaacii* and *V. klebahnii* induce any of three known plant defense pathways through the use of real-time PCR. We are in the process of designing and validating PCR primers to detect the differential expression of 9 plant defensin genes, which are involved in plant disease resistance. We will test the hypothesis that *V. isaacii* and/or *V. klebahnii* activate one or more plant resistance pathways, which would support our hypothesis that these two species may have application in biocontrol of Verticillium wilt. These genes include chitinase class II, pathogenesis-related protein 1b, ethylene response factor 3, and liopxygenase, and have previously been correlated with plant defense pathways. The experiments are in progress and no results are available yet to report.

Objective D. Determine the potential of host-directed evolution of *V. dahliae* genotypes of differential virulence from a single genotype.

The study of the evolution of individual genotypes of soilborne fungi over time has rarely been attempted. Additionally, no information is currently available on the impact of diverse genotypes of a plant host on soilborne pathogens. This information is critical to devising strategies for the long-term success of breeding efforts targeting diseases such as Verticillium wilt. Race 1-infected transplants of the cultivar Salinas were produced in a growth chamber, transplanted into the fumigated field at the USDA Station where they were grown until maturity, and incorporated by tillage. This field had no history of Verticillium wilt on lettuce. This infestation was performed over two growing seasons. This field currently serves as a Verticillium wilt screening nursery. In screening trials performed over the past two seasons, all race 1-susceptible lettuce cultivars and breeding lines planted in this plot developed typical Verticillium wilt. It may be safe to claim that a single genotype of *V. dahliae* was introduced into the field. Since the breeding lines also included some with differential susceptibility to Verticillium wilt, it is likely that they exerted selection pressure on the resident pathogen population. Over the successive lettuce cropping cycles in this field plot, we plan on collecting samples from each lettuce season and genotype them using the microsatellite markers that we have developed. This will allow us to study the evolution of this individual genotype in the natural environment, but most importantly it will provide us with insight into the impact of lettuce on this evolution. Does the host apply selective pressure on *V. dahliae* leading to its genotypic diversification? In the situation where the host does apply a selection pressure, then it is likely that a monoculture of one host genotype will lead to a rapid diversification of the pathogen and an increase in pathogen fitness. Subsequently, this may mean that using near-isogenic lines carrying the gene(s) coding for resistance may be preferable to provide long-term success. Alternatively, if the host applies

little or no selection pressure, the expectation is that the pathogen's genotype that was introduced in the field plot will remain unchanged over the successive growing seasons. This would mean that if the introduction of new genotypes is curbed, no new alleles would appear in the resident population, unless they are the result of mutation. Subsequently, breeders may expect that a monoculture of resistant lettuce cultivars is likely to be sustainable over a number of years.

Methods

Verticillium dahliae isolates were collected over four years, beginning with the 2010 field season, until 2013. These samples were collected from symptomatic lettuce cultivars and breeding lines at the time of Verticillium wilt severity ratings in the field. The samples collected were brought to the lab and washed thoroughly with tap water to remove soil and debris from the taproot. Fungi were isolated and cultured using our standard protocols. A total of 235 *Verticillium* isolates were collected using these methods and verified as *V. dahliae* using species-specific PCR. Thirteen previously developed polymorphic microsatellite markers were used to genotype samples from each year. Multilocus genotypes were compared to the known genotype of the race 1 strain Ls16, which was used to inoculate the field at the start of the experiment. Race-specific PCR was also used to screen for the presence of the race 1-specific gene, *Ave1* in all of the field isolates.

Results

Growing Season	No. Isolates collected	No. isolates with a mutant allele
2010	69	0
2011	39	0
2012	65	2
2013 1st	96	1
2013 2nd	96	2

Analyses of the first collection of samples from the first two growing seasons revealed that all samples of *V. dahliae* were genetically indistinguishable from Ls16, using the markers employed. In 2010 and 2011, 65 and 39 isolates, respectively were successfully genotyped. No allelic variations (mutations) were observed at any of the 13 loci. In 2012, two isolates out of a total 38 were observed to have a single motif polymorphism each at two different loci, indicating that some slight mutations from the original genotype used to inoculate the field had occurred. Finally, in 2013 two samples were collected in two different growing seasons. In the first growing season, 1 isolate out of 96 genotyped were observed to have microsatellite mutations. In the second season, 2 isolates out of 96 genotyped contained microsatellite mutations. Thus, over four years of sampling we observed 5 instances where changes had occurred within the microsatellite alleles.

Given that for 235 isolates, 13 loci were characterized over 5 growing seasons, this means that only 1 locus / 3055 / growing season experienced any mutation. This mutation rate is quite similar to what has been reported in human microsatellites, 10^{-3} to 10^{-4} per generation per locus.

In fact, the mutation rate of *V. dahliae* is probably lower than in humans, because the generation time is much shorter (days).

We speculate that since the microsatellites used are diverse in their motif sizes and are scattered throughout the genome of *V. dahliae*, they appropriately model the overall genome evolution process. We hypothesize that the potential for rapid evolution of *V. dahliae* in response to crop genotype is low. Crop resistance, therefore, may be expected to be highly stable for many years, because new genotypes of the fungus are slow to evolve, and sexual recombination in soilborne populations appears to be nonexistent at least in this genotype. As long as quarantine measures are taken to exclude the influx of novel pathogen genotypes, popular cultivars with resistant properties may be expected to remain resistant for long periods of time.

Mutant alleles from the isolates that differed from the parent Ls16 strain were confirmed by repeating PCR and capillary electrophoresis. These five isolates are being evaluated in the greenhouse to compare their virulence to Ls16 using standard protocols and differential lettuce lines. We suspect that the virulence of these isolates will be on par with Ls16, but there is a chance that the virulence may be either lower or higher. The first experiment will be evaluated in the next 4 weeks.

Objective E. Create a race 2-infested plot at the USDA Station.

To develop a field site infested with only race 2 strains of *V. dahliae*, a 1-acre site at the USDA Station in Salinas was identified. The field site was fumigated with methyl bromide and chloropicrin in the spring of 2011. During the summer of 2011, 12,000 seedlings of lettuce cultivar 'Salinas' were produced in greenhouse trays and inoculated by soil drench method three times before being transplanted at the fumigated field site. The crop developed very high levels of Verticillium wilt incidence. At maturity, the crop was incorporated into the soil. A second crop was transplanted in the spring of 2012 and was incorporated into the soil in July. Assuming the inoculum densities will be similar to the race 1 screening site after two inoculated crops, we planted the germplasm and breeding material for evaluation during the fall of 2012. No disease developed even in highly susceptible lines and the soil inoculum density evaluations revealed low numbers of microsclerotia. This was surprising but quite possibly due to the fact that the race 2 strain used may have a lower reproductive potential in infected plants compared with race 1. We therefore transplanted 24,000 seedlings of lettuce cv. Salinas on March 27, 2013 that had been grown in plug trays in the UC Davis greenhouse at the USDA-ARS research station in Salinas. These transplants had also been inoculated three times at weekly intervals beginning with three weeks after emergence with 3 ml of a *V. dahliae* strain Ls17 spore suspension at a concentration of 1×10^{-8} conidia per ml. The last inoculation occurred on March 20. The disease incidence and severity was still low. With this infected crop incorporated, we expected the inoculum density in soil to reach high levels required for Verticillium wilt development in lettuce but lack of disease on plants prevented this. We are therefore repeating the production of an infected lettuce crop from inoculated seedlings again in the summer of 2014.

Objective F. 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.

Methods and Results. Since the initial discovery of Verticillium wilt in lettuce in 1995, the disease has spread within the Salinas Valley. In affected fields, losses have ranged between 30 to 90%. Therefore, Verticillium wilt of lettuce caused by *Verticillium dahliae* has become a major concern to the California lettuce industry. The soilborne pathogen produces long-term resting structures called microsclerotia that remain dormant in the soil for 10 to 15 years. Fumigation is not economically feasible for lettuce, and crop rotation is ineffective due to the broad host range of *V. dahliae*. Therefore, the development of lettuce cultivars resistant to Verticillium wilt is important to the survival of the lettuce industry in California.

Funding from the National Plant Germplasm System has allowed us to screen lettuce germplasm for resistance to Verticillium wilt. Through this we identified two distinct pathogenic races of *V. dahliae* as well as resistance to race 1 isolates in diverse lettuce types. A single gene named Verticillium resistance 1 was identified in the Batavia cultivar La Brillante, and race 1 resistant iceberg breeding lines have been developed and released developed. Seed of race 1 resistant materials were deposited into the WRPIS (Western Regional Plant Introduction Station). All of this germplasm is susceptible to race 2 isolates.

The existence of race 2 isolates in California lettuce production fields is certain. Moreover, it is highly probable that widespread use of race 1 resistant germplasm will select for and increase the economic damage caused by race 2 isolates. Even more concerning is the finding that race 2 isolates can be introduced on infested seed of spinach, a crop widely grown in rotation with lettuce in the Salinas Valley. Therefore, identification and subsequent development of lettuce cultivars with resistance to race 2 is imperative for sustaining the lettuce industry.

To date, we have screened over 850 *L. sativa* and *L. serriola* accessions using race 2 *V. dahliae* isolate VdLs17. We have confirmed partial resistance (disease incidence significantly lower than 'Salinas') in four accessions (PIs 169511, 171674, 204707, 226641) (Hayes et al. 2011c). However, all of these PIs have had at least a few symptomatic plants, and all but PI 171674 have had non-symptomatic plants that are nonetheless colonized by *V. dahliae*. It does not appear that these PIs have a sufficient level of resistance to control the disease, and we are pursuing research to determine if intercrossing these four accessions will result in progeny with greater levels of resistance. Complete resistance to race 2 needs to be found, and we will continue to screen the collection in hopes of finding this trait.

We have also identified genetic variation in iceberg cultivars for the onset of foliar symptoms relative to market maturity. The cultivars Anuenue (PI 536800), Batavia Reine des Glaces (PI 634668), Climax (PI 536705), Desert Storm (no PI number), and Bubba (no PI number) appear to delay foliar wilting past market maturity despite vascular root colonization by *V. dahliae*. Iceberg cultivars with delayed foliar symptoms may be useful for reducing crop losses from Verticillium wilt. Initial research was conducted using field sites infested with race 1 and additional research is needed to confirm these results and to determine if these cultivars exhibit delayed foliar symptoms against both races.

Objective G. Determine the effects of temperature on Verticillium wilt development in lettuce.

Temperature plays an important role in the interaction between lettuce plants and *Verticillium* isolates. Knowledge of the role of temperature in the development of the disease would provide

important epidemiological information on this disease and provide a rationale for why we observe higher incidence and severity on lettuce crops during the fall season. We have observed variation in the *in vitro* growth rates of several isolates, within *V. dahliae* and *V. isaacii* and *V. klebahnii*. While there do not appear to be any fixed differences between species or pathogenic races of *V. dahliae*, all isolates respond differently to temperatures tested (20° C, 25° C, 30° C). We hypothesized that there is an optimal temperature for *V. dahliae* to colonize and cause severe symptoms within lettuce and was tested in this study.

Methods

We inoculated the susceptible Salinas cultivar with GFP-tagged, race 1 and 2 isolates Ls 16 and Ls 17, respectively, and grew the plants in three temperature-controlled growth rooms (20°, 25° and 30° C) and growth chambers and monitored both the severity of the disease and the growth of the pathogen within the vascular system over the course of 24 h, 48 h, 1, 2, 4, 6, and 8 weeks post-inoculation. The samples was taken to Davis to examine them under confocal microscope to determine if the temperature variation significantly alters the development of Verticillium wilt.

Results

Confocal microscopy observations showed that extensive root colonization can occur at all three temperatures but more so at 20° C (Figure 1).

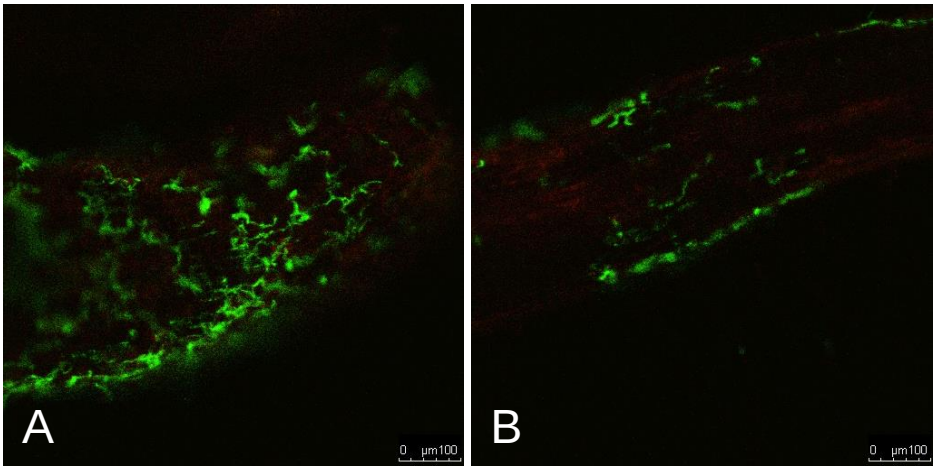

Figure 1. Comparison of *V. dahliae* root colonization (external root surface) seen at A. 20° C air temperature and B. 30° C air temperature at 15 days post inoculation.

However, this research has been hampered by lack of available growth chambers at the Salinas station. Further work on this has been postponed until growth chambers can be repaired or shared with USDA-ARS personnel. Future work will evaluate disease severity at the end of 8 weeks of continual plant growth in temperature-controlled environments.