CALIFORNIA LETTUCE RESEARCH PROGRAM

April 1, 2011 - March 31, 2012

BIOLOGY AND EPIDEMIOLOGY OF VERTICILLIUM WILT OF LETTUCE

Krishna V. Subbarao
Department of Plant Pathology
University of California, Davis

SUMMARY

There were five objectives during the current funding cycle and included: a) to determine whether or not nitrate fertilizers used in strawberries following fumigation are a factor in rendering soil Verticillium wilt-conducive relative to ammoniacal fertilizers; b) to determine the potential of host-directed evolution of *V. dahliae* genotypes of differential virulence from a single genotype; c) to screen germplasm collected from Armenia and Georgia against a range of race 2 strains of *Verticillium dahliae*; d) to create a race 2-infested plot at the USDA Station; and e) to continue the breeding program to identify and develop race 1 resistance in crisphead, leaf, and other lettuce types. Fertilization practices on strawberries planted post-fumigation are suspected to be a factor in the rapid re-colonization of soil by *V. dahliae* microsclerotia and result in rapid high wilt incidence in lettuce crops that follow strawberry. We conducted two experiments in the greenhouse to ascertain the role of NO_3^- and NH_4^+ fertilizers on Verticillium wilt of lettuce. Results from both experiments were consistent and suggested that NH_4^+ fertilizer resulted in significantly higher wilt incidence and severity contrary to the literature. At the fertilizer rates used on lettuce, little change in pH occurred with NH_4^+ and hence the lack of disease suppression with the ammoniacal fertilizers. Thus, the perception that nitrate fertilizers used in strawberries would render the soil conducive to Verticillium wilt on lettuce could not be verified in our studies. Additional isolates to determine the host-directed evolution of *V. dahliae* were collected and analyses of all isolates has begun. We screened the germplasm collected from Armenia and Georgia against race 2 isolates in the greenhouse in Salinas. While some lines differed in the levels of disease developed, none of them could be characterized as resistant to race 2. These results were inconsistent with those obtained in screenings done in Davis. We therefore obtained the selections made in Davis to screen for resistance in Salinas, which is currently in progress. We also created a one-acre area infested with race 2 last year and with another inoculated crop of a susceptible cultivar in 2012, this field should be available for field screening against race 2. Additional breeding lines were screened for resistance to race 1 in the field and in the greenhouse.

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

PRINCIPAL INVESTIGATOR: **Krishna V. Subbarao**
Department of Plant Pathology
University of California, Davis

COOPERATING PERSONNEL: **Karun Maruthachalam, Patrik Inderbitzin, Suraj Gurung, and Dylan Short**
Department of Plant Pathology,
University of California, Davis

Steven T. Koike
U. C. Cooperative Extension, Salinas, CA

Ryan Hayes
USDA-ARS, Salinas

OBJECTIVES:

A. Determine whether or not nitrate fertilizers used in strawberries following fumigation are a factor in rendering soil Verticillium wilt-conducive relative to ammoniacal fertilizers.
B. Determine the potential of host-directed evolution of *V. dahliae* genotypes of differential virulence from a single genotype.
C. Screen germplasm (*L. serriola, L. georgica, L. virosa* in the order of priority) collected from Armenia and Georgia against a range of race 2 strains of *Verticillium dahliae*.
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.

PROCEDURES AND RESULTS:

Objective A. Determine whether or not nitrate fertilizers used in strawberries following fumigation are a factor in rendering soil Verticillium wilt-conducive relative to ammoniacal fertilizers.

Methods. Experiments were conducted in a greenhouse to determine the effect of three common fertilizers that are commonly used in lettuce production to supply nitrogen either as ammonium or

nitrate or a combination of the two on Verticillium wilt of lettuce. The fertilizers used were ammonium sulfate, ammonium nitrate and calcium nitrate. Experiment was laid out in a completely randomized design with five replications per treatment and five plants within each replication. Lettuce plants of cv. Salinas were raised in one gallon plastic pots containing sterilized sand. Hoagland solution containing all the essential nutrients excluding nitrogen was applied three times a week to each pot. Plants were thinned to retain a single plant per pot three weeks after emergence. Nitrogen treatments were applied from the third week after planting. Ammonium sulfate, ammonium nitrate and calcium nitrate solutions were prepared in deionized water and applied to corresponding treatments at a rate of 60 lb N per acre three times at two week intervals. *Verticillium dahliae* conidial suspension (from the race 2 strain LS 17) prepared from 7 to 8-day-old cultures was added to the soil (3 ml/pot @10^6 conidia/ml) root in each pot. Plants were inoculated twice at 4 and 6 weeks after planting. Pots without *V. dahliae* served as non-inoculated control. This experiment was conducted twice.

The levels of NO_3 and NH_4 ions in soil were determined immediately after N application and 3 and 6 days after each N application. As there were five pots in each replication in each treatment, one soil core was collected from each of the five pots using a 2-cm-diameter soil sampler at about 4 cm depth, placed in a canister and mixed thoroughly. Nitrogen extractions were made in 2M KCL following the standard protocol and shipped at -20 C to the UC Davis analytical laboratory, where concentrations of NO_3 and NH_4 were determined.

Results

The analysis of variance revealed that interactions between treatments and experiments were not significant ($P = 0.637$) and therefore, data from the two experiments were combined and analyzed. Combined analysis revealed that there was a significant difference among treatments. Disease incidence in the ammonium sulfate treatment was significantly higher than in both ammonium nitrate and calcium nitrate treatments (Fig. 1). Disease incidence in the two NO_3 nitrogen treatments (ammonium nitrate and calcium nitrate) was not significantly different (Fig. 1). Disease severity in the ammonium sulfate treatment was also significantly higher than in ammonium nitrate and calcium nitrate treatments (Fig. 2). The levels of NH_4 and NO_3 were highest immediately after the fertilizer application and decreased progressively by 7 days in all treatments (Fig. 3). The perception that nitrate fertilizers used in strawberries would render the soil conducive to Verticillium wilt on lettuce could not be verified in our studies.

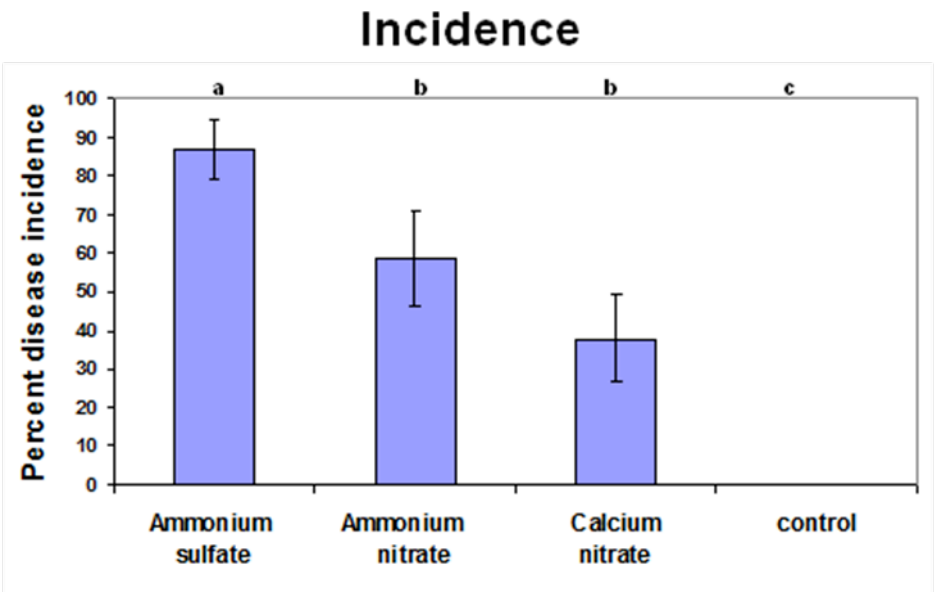

Figure 1. Effects of different forms of nitrogen on Verticillium wilt incidence in lettuce in greenhouse experiments. Different letters above the columns suggest significant differences between treatments according to Tukey's test (P<0.05).

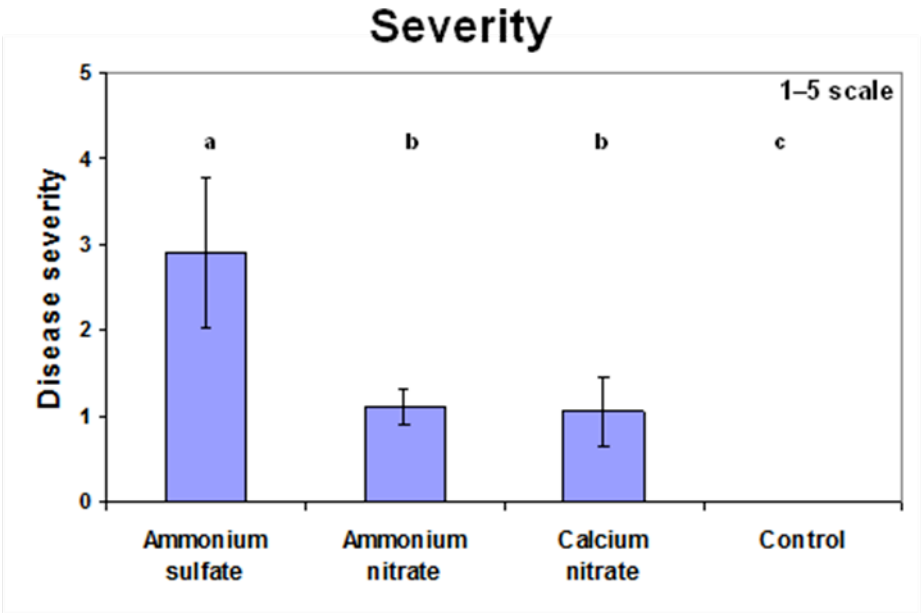

Figure 2. Effects of different forms of nitrogen on severity of Veriticillium wilt in greenhouse experiments. Different letters above the columns suggest significant differences between treatments according to Tukey's test (P<0.05).

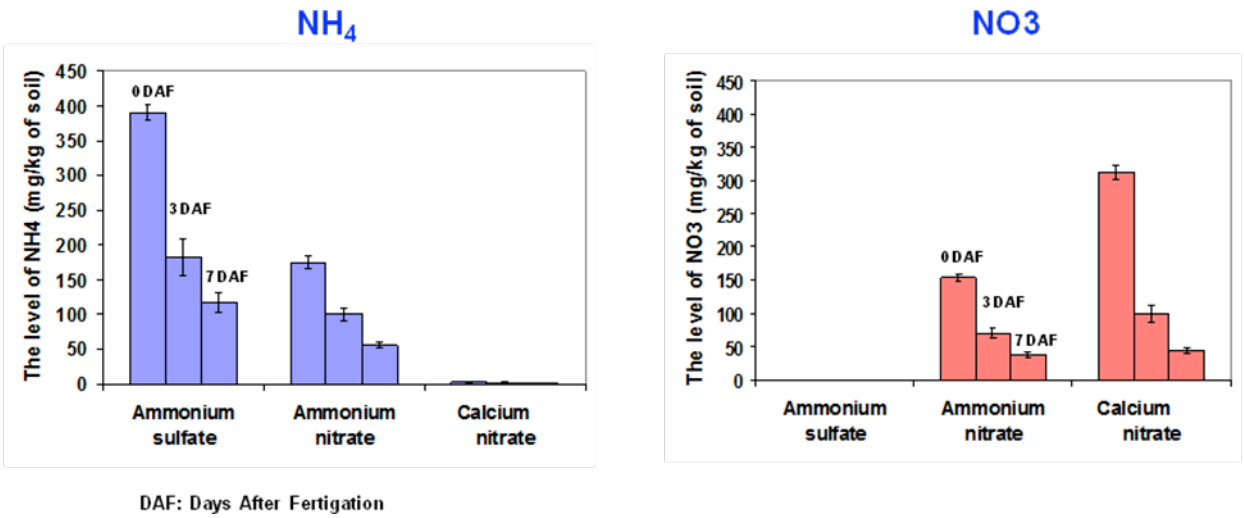

Figure 3. The NH_4 and NO_3 levels in soil following fertigation. Applications of N were made 3 weeks after seeding, and NH_4 and NO_3 levels were determined at three times; 0, 3, and 7 days of application. N was applied three times during the experiment. The values for each period are the average of values from three N applications.

Objective B. 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 fungi over time has not 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. The 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 and race 1 infestation was performed over two growing seasons. This field is currently serving as a Verticillium wilt screening nursery for the past three years. 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 quite likely that they exerted selection pressure on the resident pathogen population. Over the successive lettuce cropping cycles in this field plot, we have been 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 (race 1) 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 its fitness. Subsequently, this may mean that using near-isogenic lines carrying the gene(s) coding for resistance may be preferable to provide for a long-term success. Alternatively, if the host applies little or no selection pressure, the expectation is that the 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 it is induced by mutation. Subsequently, breeders may expect that a monoculture of resistant lettuce cultivars is likely to be sustainable over a number of years.

Methods. During the course of the evaluation of the breeding material in the spring and summer seasons of 2010, 140 and 120 stems, respectively, and during the summer season of 2011, 91 stems were collected from symptomatic lettuce plants. The stem pieces were plated on NP-10 medium to isolate *V. dahliae*. A total of 76 and 40 isolates were collected, purified, and stored from spring and summer seasons of 2010, respectively. From 2011, we have collected 41 additional isolates. We have thus far analyzed 46 isolates from the spring season using the recently developed race 1-specific primers, which confirmed 43 isolates as race 1. The analyses of remaining isolates are in progress.

Objective C. Screen germplasm (*L. serriola, L. georgica, L. virosa* in the order of priority) collected from Armenia and Georgia against a range of race 2 strains of *Verticillium dahliae.*

Germplasm lines from the *Lactuca* species collected from Armenia and Georgia screened in Salinas were susceptible to race 1 isolate of *V. dahliae*. These results are at variance with the results obtained by Michelmore's group in Davis. Since plants obtained from seed from different aliquots were screened at the two locations, we have obtained the selections from Davis that exhibited various degrees of resistance for screening in Salinas, which is currently in progress.

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

To have a field site infested with only the race 2 strains of *V. dahliae* available, 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 has been transplanted in the spring of 2012 and will be incorporated into the soil in July. The field site is expected to be ready for screening of germplasm and breeding material by fall 2012.

Objective E. 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 resistance 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.

We are currently screening the Western Regional Plant Introduction Station (WRPIS) *Lactuca* collection for resistance to race 2 isolates of *V. dahliae*. More specifically, our research is using a working collection of the WRPIS located in Salinas, CA. Our strategy uses greenhouse testing to screen up to eight plants of 160 accessions per year in unreplicated plots to identify candidate sources of resistance. This is followed by replicated greenhouse and field-micro-plot experiments to confirm resistance. Verticillium wilt disease development in lettuce is dependent on plant development, and in some genotypes symptoms are not expressed until the plant reaches flowering. We maintain the plants in the greenhouse until flowering begins, at which time disease evaluations are conducted. This substantially lengthens the duration of each experiment, but is necessary to reduce the number of false positives. At all stages of testing, crown sections of asymptomatic plants are plated on NP10 media to determine the presence / absence of *V. dahliae* stem infection. To date, we have screened 824 *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). However, all of these PIs have had at least a few symptomatic plants, and all but PI171674 have had non-symptomatic plants that are nonetheless colonized by *V. dahliae*. Complete resistance to race 2 has not yet been found, and we will continue to screen the collection in hopes of finding this trait. This includes new *L. serriola* accessions collected through the efforts of the USDA, Plant Exchange Office. Results from this research were published.