Project title: The effects of planting date, varietal susceptibility and residue management on severity of Fusarium wilt in lettuce

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Abstract

Fusarium wilt of lettuce, caused by *Fusarium oxysporum* f. sp. *lactucae*, is a serious disease affecting lettuce production in most major growing districts in California and Arizona. The disease is most problematic in warmer areas, which includes the San Joaquin Valley but also coastal locations in the vicinity of King City and points south. To determine if the effect of temperature on disease severity can provide a basis for minimizing damage caused by Fusarium wilt, lettuce cultivars differing in susceptibility to this disease were transplanted into an infested field on three different dates. Five weeks after planting most plants of the two most susceptible cultivars had died from the disease, whereas plants of the resistant leaf cultivar, Fossey, were either healthy or showed only mild symptoms. Planting date had only a very small effect on these three cultivars. However, for three iceberg cultivars of intermediate susceptibility, disease was significantly more severe in the first planting than in the second or third planting windows. The differential development of disease was associated with higher temperatures during the second and third planting windows, relative to the first. These results were consistent with what was observed when the same experiment was conducted in 2014. Inoculum levels are another important determinant of disease and consequently, minimizing the build-up of inoculum in soil is an essential component of disease management. During 2015 we completed experiments designed to determine if drying crop residue on the soil surface prior to incorporation will influence pathogen survival. The results of both experiments showed that after one year, there was not a significant difference in soil inoculum densities between plots in which residue was incorporated immediately and those in which residue was dried before it was incorporated. These findings are consistent with the results of experiments conducted in 2014. Although all crisphead cultivars are susceptible to Fusarium wilt, the extent to which disease develops will be influenced both by inoculum levels and prevailing temperature during the growing season. Experiments conducted during the past year documented that two crisphead cultivars (Diamondback and Steamboat) remained free of above-ground disease symptoms or nearly so when planted in October or April in soil with 104 colony forming units or less of *F. o.* f. sp. *lactucae*. Incorporation of chitin at a rate of approximately 0.1% (w/w) did not reduce the severity of Fusarium wilt compared to unamended soil.

Objectives

1. Complete data collection for experiments to assess the effect of drying crop residue prior to incorporation on survival of the Fusarium wilt pathogen.

2. Determine if the chitin amendments can inhibit the activity of the Fusarium wilt pathogen and reduce severity of disease.

3. Conduct a second-year planting date experiment to assess the effect on severity of Fusarium wilt.

4. Characterize differences in susceptibility of crisphead cultivars at low and intermediate inoculum levels.

Procedures

Objective 1

Infected lettuce plants were obtained from an experimental field on the Davis campus that is infested with the Fusarium wilt pathogen. Plants harvested from this field were transferred to microplots (2' x 2') and either incorporated immediately or spread over the soil surface and allowed to dry for eight days prior to incorporation. Each treatment (incorporated immediately or not) was represented by five plots. Thirteen days after incorporation of the dried residue, soil in all plots was mixed using a hand-held cultivator. At one, two, three, six, nine and 12 months after incorporation of the dried residue, soil cores were removed from each plot and assayed to determine the state of decomposition of the residue and the density of inoculum. Inoculum density was estimated using soil dilution plating. A composite of multiple soil cores was taken from each replication and a sub-sample was suspended in water. Various dilutions of this suspension were spread over the surface of plates containing a *Fusarium*-selective medium. Colonies corresponding to the Fusarium wilt pathogen were enumerated seven days later. This experiment was initiated in August of 2013 and again in October of 2013. The results of these experiments were reported previously. To better understand the extent to which seasonal factors may influence results, the same experiment was initiated also in April of 2014 and again in June of 2014. As before, soil populations of *F. o. lactucae* were monitored over a twelve month period following incorporation of crop residue.

Objective 2

Soil from an experimental field on the Davis campus that is naturally infested with the Fusarium wilt pathogen was blended with sand at a ratio of 1:1 (volume:volume) to improve drainage. Soil was either un-amended or supplemented with 0.1% Rootguard (weight/weight). Rootguard is a combination of crab meal and feather meal, with a high concentration of chitin. Following incorporation of Rootguard, the mix was maintained at field capacity for eight days. During that time soil was mixed daily to prevent it from becoming anaerobic. Thereafter, soil was dispensed into 4" diameter pots, and one 4 week-old seedling (cultivar Sidewinder) was transplanted into each pot. Plants were maintained in a growth chamber set for day/night temperatures of 30/22 °C, with a 14 hour photoperiod. An experiment included 40 pots for each treatment, with one plant per pot, and the experiment was conducted twice. Plants were rated for disease severity at weekly intervals on a 1-4 scale, with 1 corresponding to a healthy plant and 4 given to a plant killed by Fusarium wilt. A rating of 2 was given to plants that were stunted but not otherwise symptomatic, and a rating of 3 corresponded to severe stunting with yellowing and/or death of some leaves. Inoculum density of the soil was quantified at the start and end of the experiment using soil dilution planting, as described under objective 1. The frequency with which roots became infected was estimated by placing washed roots on a Fusarium selective medium and counting the number of points at which colonies of the Fusarium wilt pathogen emerged. At the conclusion of the experiment (11 weeks post-transplanting), plants were rated for disease severity on a 1-5 scale, based on the extent of discoloration of the tap root, with 1 corresponding to a healthy appearance and higher numbers corresponding to greater degrees of reddish-brown coloration; a rating of 5 was given to a dead plant. After the final rating, the above-ground portion of each plant was weighed after drying at 30 °C for four days.

Objective 3

Seven cultivars were transplanted on each of three dates (20 August, 27 August and 3 September) into a naturally infested field on the UC Davis Plant Pathology Department research farm. Plants had been grown in a greenhouse for approximately five weeks prior to transplanting. The cultivars used in this experiment were: ‘Fossey’, a leaf type known to be resistant to Fusarium wilt, one romaine cultivar, ‘Bondi’, and five crisphead cultivars: ‘Diamondback’, ‘Gabilan’, ‘Salute’, ‘Sidewinder’ and ‘Steamboat’. Cultivars were selected based on performance in past trials and suggestions from industry representatives. The field was divided into six equally sized blocks, and all planting dates of all cultivars were included in each block to minimize effects of heterogeneity of inoculum levels within the field. Accordingly, there were six replications of each cultivar at each planting date. A replication consisted of ten plants that were transplanted into a bed with one foot spacing between plants. A stand count was taken one week after planting, and ratings for disease symptoms were conducted at three, four, five and six weeks after planting. Plants were rated for disease severity on a 1-4 scale, as described above. This was a repeat of an experiment conducted during 2014.

Objective 4

Procedures for this experiment were the same as described for objective three except that only two cultivars were used (Diamondback and Steamboat) and plants were grown at a different location on the UC Davis Plant Pathology Department research farm. The experiment was initiated by direct seeding on October 13, 2015 and plants were rated at intervals thereafter on the 1-4 scale described above. The final rating was 15 weeks later. At this time, plants were also rated based on tap root symptoms, as described above. A second experiment using the same cultivars was initiated on April 6, 2016 and a final rating was taken seven weeks later. In both experiments, replicate plantings of both cultivars were established in each of six blocks.

Results and Discussion

Residue treatment experiments where conducted in soil that was initially free of the pathogen. In the first experiment (initiated in April of 2014), inoculum levels at one month after incorporation dropped more rapidly, on average, in plots in which residue was allowed to dry on the soil surface prior to incorporation. Thereafter, inoculum densities changed very little and there was not a significant difference between the two treatments at any assessment interval (Figure 1).

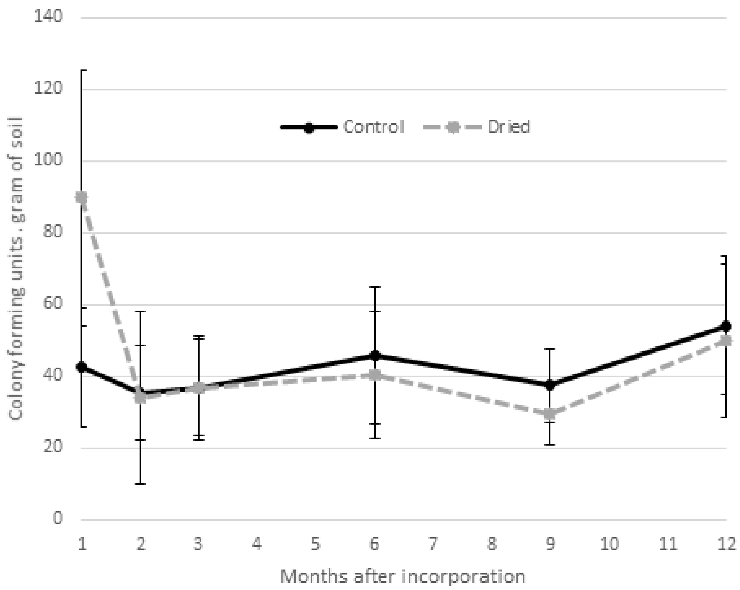


Figure 1. The density of inoculum of *Fusarium oxysporum* f. sp. *lactucae* in soil at intervals after incorporation of infested crop residue in April of 2014. Residue was either incorporated immediately or after drying on the soil surface. Error bars correspond to the standard error of the mean.

For the experiment initiated in June of 2014, inoculum levels were initially lower in the dried residue plots and this differential remained throughout the 12 month monitoring period (Figure 2). Based on the rate at which inoculum levels declined, there was not a significant difference between the two treatments.

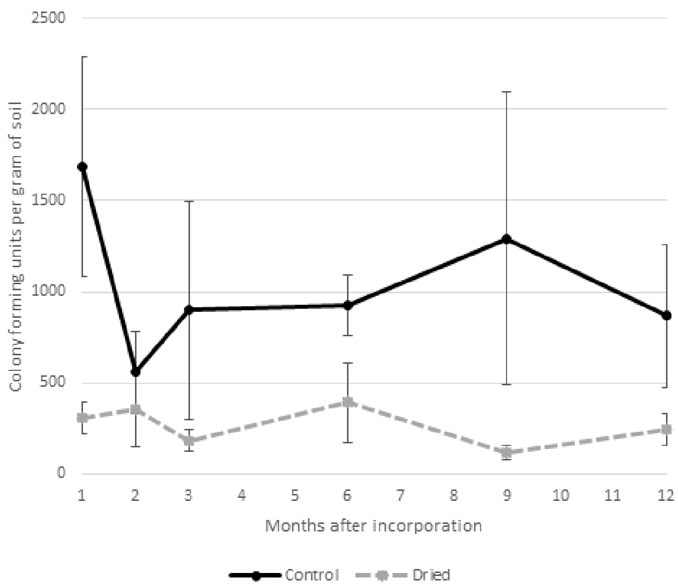


Figure 2. The density of inoculum of *Fusarium oxysporum* f. sp. *lactucae* in soil at intervals after incorporation of infested crop residue in June of 2014. Residue was either incorporated immediately or after drying on the soil surface. Error bars correspond to the standard error of the mean.

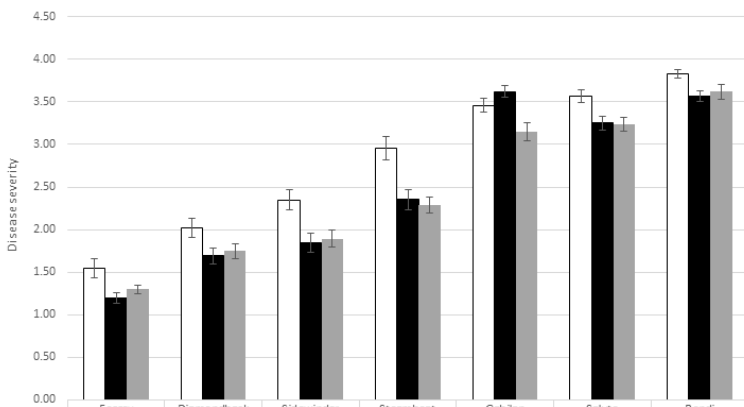
For the first test of Rootguard, the initial inoculum density was approximately 730 colony forming units per gram of soil. By the end of the experiment, inoculum density was lower in the chitin amended soil than in the control (Table 1). There was not a significant effect of treatment on the frequency with which roots became infected but disease was more severe in chitin amended soil, based on both above- and below-ground symptoms (Table 1). Plants achieved a significantly greater dry weight in chitin amended soil (Table 1). The second experiment has not yet been completed but based on above ground symptoms at seven weeks post-transplanting, disease was more severe in chitin amended soil (severity rating = 1.85 ± 0.16) than in un-amended soil (1.11 ± 0.05).

Table 1. Effects of chitin amendment in soil on Fusarium wilt of lettuce

Treatment[1]	Disease severity[2]		Root infections[3]	Dry weight[4]	Soil ID[5]
	Shoot	Tap root			
Chitin	2.76 ± 0.20	3.78 ± 0.22	0.20 ± 0.05	0.61 ± 0.06	698 ± 65
Control	1.91 ± 0.15	1.90 ± 0.22	0.13 ± 0.03	0.30 ± 0.02	906 ± 72

[1]Soil was amended with Rootguard at 0.1% (= chitin) or not (= control)
[2]Disease severity was based on above-ground (= shoot) and below-ground (= tap root) symptoms; entries correspond to the average ± standard error
[3]Number of infections per centimeter of root; entries correspond to the average ± standard error
[4]Weight of the above-ground portion of the plant; entries correspond to the average ± standard error
[5]The number of colony forming units of the Fusarium wilt pathogen per gram of soil at the conclusion of the experiment; entries correspond to the average ± standard error

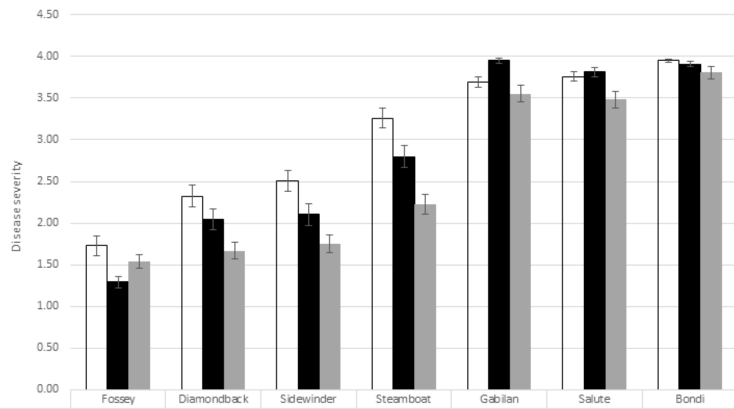
The results of the planting date experiment were very similar to what was observed in an experiment of the same design conducted in 2014. Differences in disease severity between cultivars were apparent by four weeks after planting. Bondi was the most susceptible, with average ratings of 3.46 ± 0.08, 3.12 ± 0.11 and 2.51 ± 0.10 for the first, second and third planting dates, respectively. Fossey was the least affected, with most plants appearing healthy at all three planting dates and only mild stunting in a few plants in each of the three plantings. The remaining five cultivars had intermediate disease severity ratings. Differences between cultivars were still evident at five weeks after planting (Figure 3). In all cases, disease was less severe in the third planting than in the first. This effect of planting date was strongest for cultivars of intermediate susceptibility: Sidewinder and Steamboat (Figure 3). Disease severity in the second planting was very similar to the third for all cultivars except Gabilan, for which disease was most severe in the second planting (Figure 3).



	Fossey	Diamondback	Sidewinder	Steamboat	Gabilan	Salute	Bondi
1st Planting Date	1.54	2.02	2.35	2.96	3.46	3.57	3.83
2nd Planting Date	1.20	1.69	1.85	2.36	3.62	3.25	3.57
3rd Planting Date	1.30	1.75	1.89	2.29	3.15	3.24	3.62

Figure 3. Disease severity in seven lettuce cultivars at five weeks after planting on either 20 August (first planting), 27 August (second planting) or 3 September (third planting). Error bars correspond to the standard error of the mean.

By the final rating at six weeks, plants of cultivars Gabilan, Salute and Bondi were severely diseased or dead, with little difference between planting dates. In contrast, significant differences between planting dates were evident for cultivars of intermediate susceptibility: Diamondback, Sidewinder and Steamboat. For these three cultivars, disease severity was highest in the first planting, lowest in the third and intermediate in the second (Figure 4).



	Fossey	Diamondback	Sidewinder	Steamboat	Gabilan	Salute	Bondi
1st Planting Date	1.73	2.32	2.51	3.26	3.69	3.76	3.95
2nd Planting Date	1.29	2.04	2.10	2.80	3.95	3.81	3.91
3rd Planting Date	1.54	1.67	1.75	2.23	3.55	3.48	3.81

Figure 4. Disease severity in seven lettuce cultivars at six weeks after planting on either 20 August (first planting), 27 August (second planting) or 3 September (third planting). Error bars correspond to the standard error of the mean.

Lower disease severity in the second planting, compared to the first, was associated with higher temperatures between 19 and 23 days after planting for the first compared to the second planting (Figure 5). A similar but less pronounced temperature differential was evident between the first and third planting dates (Figure 6). This pattern is consistent with the expectation that Fusarium wilt would be more severe under warm conditions, and, as was observed in the 2014 experiment, it appears that the third week after planting may be the most critical period for temperature effects on disease.

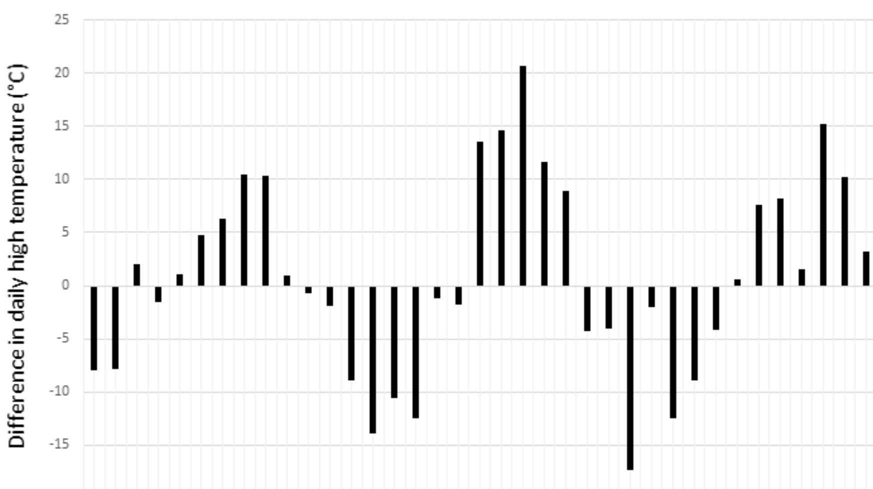


Figure 5. The difference in daily high temperature between the first and second planting dates. A positive value indicates temperature was higher following the first planting date.

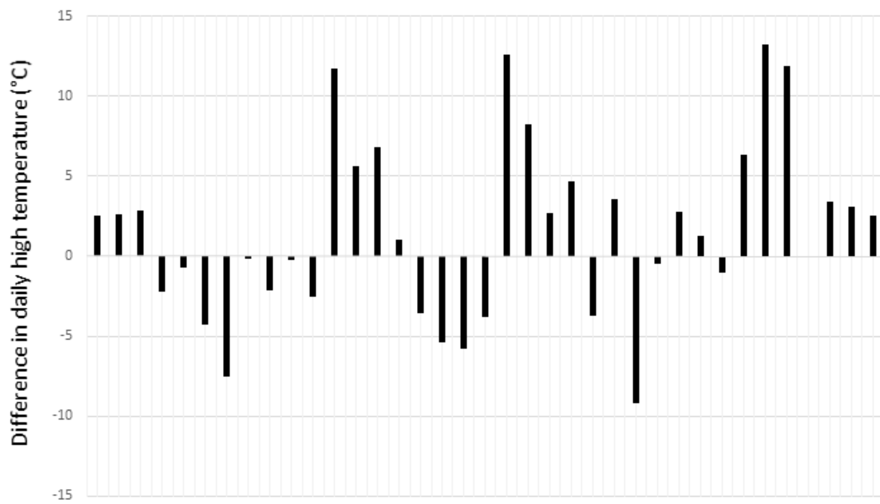


Figure 6. The difference in daily high temperature between the first and third planting dates. A positive value indicates temperature was higher following the first planting date.

Two crisphead cultivars that were rated as intermediate in susceptibility to Fusarium wilt in experiments described above (Diamondback and Steamboat), were tested in a field where inoculum densities of *F. o. lactucae* ranged from less than 20 colony forming units per gram of soil to 104 colony forming units per gram. For the October planting, all plants of both cultivars appeared completely healthy based on above-ground symptoms, with only mild discoloration evident on tap roots of a few plants (Table 2). Daily high temperatures during this experiment ranged from 6 to 31 °C, with a mean of 15.8 °C. The highest temperatures occurred soon after seeds were sown, with a maximum daily high of only 23 °C for the period beginning 21 days after sowing. Much warmer temperatures prevailed following the planting in April, when daily high temperatures ranged from 16 to 40 °C with a mean of 27 °C. Even so, most plants were scored as healthy based on above ground symptoms at the final rating (Table 2). However, discoloration of the tap root was evident in more plants than in the first experiment (Table 2), which presumably reflects the influence of warmer temperatures. The results of both experiments support the conclusion that cultivars known to be susceptible to Fusarium wilt based on standard tests can be grown without significant damage from this disease at low inoculum levels.

Table 2. Disease severity in crisphead cultivars grown in soil infested with *Fusarium oxysporum* f.sp. *lactucae*.

Cultivar[1]	Block[2]	Soil ID[3]	Disease severity[4]			
			Experiment one[5]		Experiment two[6]	
			Shoot	Tap root	Shoot	Tap root
Diamondback	1	< 20.0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.06 ± 0.04
Diamondback	2	< 20.0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.08 ± 0.05
Diamondback	3	104	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.12 ± 0.07
Diamondback	4	< 20.0	1.00 ± 0.00	1.06 ± 0.06	1.00 ± 0.00	1.22 ± 0.10
Diamondback	5	20	1.00 ± 0.00	1.03 ± 0.03	1.09 ± 0.09	1.10 ± 0.05
Diamondback	6	20	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.11 ± 0.08
Steamboat	1	< 20.0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.06 ± 0.04
Steamboat	2	< 20.0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.04 ± 0.03
Steamboat	3	104	1.00 ± 0.00	1.00 ± 0.02	1.00 ± 0.00	1.61 ± 0.18
Steamboat	4	< 20.0	1.00 ± 0.00	1.02 ± 0.06	1.00 ± 0.00	1.05 ± 0.04
Steamboat	5	20	1.00 ± 0.00	1.06 ± 0.05	1.10 ± 0.10	1.38 ± 0.12
Steamboat	6	20	1.00 ± 0.00	1.10 ± 0.00	1.00 ± 0.00	1.18 ± 0.08

[1]Crisphead cultivars used in the experiments
[2]Section of the field in which lettuce was grown
[3]Number of colony forming units of the Fusarium wilt pathogen per gram of soil
[4]Disease severity was based on above-ground (= shoot) and below-ground (= tap root) symptoms; entries correspond to the average ± standard error
[5]Experiment one was initiated in October of 2015
[6]Experiment two was initiated in April of 2016