Project title: Management of Fusarium wilt through genetic resistance and manipulation of the microbial community in soil

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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. Our previous work has shown that planting dates differing by as little as one week can result in significant differences in the extent to which Fusarium wilt develops, even where inoculum levels are very high. In 2016 we extended these results by testing susceptible lettuce cultivars in soil with inoculum levels closer to what is found in commercial fields. The results showed that with inoculum levels of 104 colony forming units per gram or less, there were no aboveground symptoms under cool conditions (mean daily high and low temperatures of 27.0 and 10.6 °C, respectively). Only moderate damage was evident when the same cultivars were grown under warmer conditions (mean daily high and low temperatures of 32.8 and 12.3 °C, respectively). Results of our research in 2016 documented that incorporation of broccoli residue can significantly reduce population levels of the Fusarium wilt pathogen in soil. A commensurate reduction in disease severity was not observed, which can be attributed to the fact that our initial inoculum levels were very high. We did not find any benefit of a chitin amendment alone, and combining chitin with broccoli residue did not have a greater effect than broccoli alone. Preliminary results indicate that prior exposure to a nonpathogenic strain of *Fusarium oxysporum* may enhance resistance to Fusarium wilt but confirmation of this effect at higher inoculum levels is needed. Our previous work revealed differences in the extent of taproot colonization between cultivars that appear equally resistant based on above-ground symptoms. In 2016 we showed that recombinant inbred lines derived from a cross between leaf and romaine cultivars displayed a nearly continuous range of variation in taproot colonization. A follow-up experiment to assess the heritability of these differences is planned for 2017.

Objectives

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

2. Determine if incorporation of chitin can reduce the frequency of root infection and severity of disease caused by *F. oxysporum* f. sp. *lactucae*

3. Determine if resistance induced by non-pathogenic fungi can delay development of Fusarium wilt

4. Test recombinant inbred lines with resistance to Fusarium wilt to determine the extent to which they differ in colonization of taproots

Procedures

Objective 1

Tests of susceptibility were conducted in infested soil on the Davis campus. The infestation was established by incorporating lettuce crop residue from our planting date experiments. This was done in fall of 2013 for the purpose of testing the effects of drying residue prior to incorporation on survival of the pathogen in soil. On completion of those experiments, residue management plots were combined through cultivation of the soil and a gradient in inoculum densities was established, ranging from < 20 colony forming units (CFUs) per gram of soil to 104 CFUs per gram of soil. Inoculum density was determined using soil dilution plating. By this method soil was suspended in 0.1% water agar and dilutions were spread over the surface of plates of Komada's selective medium (KM). Plates were incubated at room temperature under continuous fluorescent light for 5-7 days, and colonies of *F. oxysporum* f. sp. *lactucae* were identified based on their distinctive colony morphology. Two crisphead cultivars of intermediate resistance to Fusarium wilt (Diamondback and Steamboat) were planted in spring and summer of 2016. In each case, crops were grown to maturity and rated periodically for severity of disease 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. At the end of each experiment, plants were also rated on a 1-5 scale based on internal discoloration in the tap root, with 1 corresponding to a healthy appearance and higher numbers corresponding to greater degrees of reddish-brown coloration.

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, supplemented with broccoli residue at 10% (weight/weight), or supplemented with broccoli at 10% (weight/weight) and 0.2% Rootguard (weight/weight). Broccoli residue consisted of stalks, branches and leaves collected from a commercial field in Salinas and chopped into pieces that were approximately 2" × 2". Rootguard is a combination of crab meal and feather meal, with a high concentration of chitin. Following incorporation of amendments, each mix was watered and thereafter maintained at field capacity for 31 days. During that time soil was mixed daily to prevent it from becoming anaerobic. Thereafter, soil was dispensed into 4" diameter pots, and one five week-old seedling (cultivar Steamboat) was transplanted into each pot. There were 20 pots in each treatment. Plants were maintained in a growth chamber set for day/night temperatures of 28/20 °C, with a 12 hour photoperiod. The experiment was conducted twice. Plants were rated for disease severity at weekly intervals on the 1-4 scale described above. Inoculum density of the soil was quantified after the 31 day incubation period (prior to transplanting) using soil dilution planting, as described under objective 1. The frequency with which roots became infected was estimated by placing washed roots on plates of KM and counting the number of points at which colonies of the Fusarium wilt pathogen emerged. At the conclusion of the experiment, plants were rated for disease severity based on the extent of discoloration of the tap root, as described under objective 1. After the final rating, the above-ground portion of each plant was weighed after drying at 40 °C for four days.

The effect of chitin on development of Fusarium wilt was also tested in a field experiment. This experiment included two treatments: 1) chitin (in the form of RootGuard) at 0.16% (weight/weight) supplemented with nitrogen in the form of broiler litter, and 2) broiler litter only, to provide the same level of nitrogen as the chitin treatment. Following incorporation of amendments, soil was irrigated and maintained at field capacity for three weeks. Unamended soil served as a control. Two cultivars were tested: Bondi and Sidewinder, each of which was transplanted into six blocks, within which all three treatments were included. Each cultivar was represented by ten plants in each block. At the conclusion of the experiment, plants were rated for disease severity based on both above-ground and tap root symptoms, as described above.

Objective 3

For objective three, we used a non-pathogenic isolate of *Fusarium oxysporum* that has been shown to induce resistance to Fusarium wilt in tomato. This isolate was grown on plates of potato dextrose agar, from which spores were collected and added to potting mix at the rate of 2.5 mls per planting cell. This was done immediately after seeding and again one week later. The inoculum density of the suspension was 1.2 * 10^6 spores per ml. Two lettuce cultivars, Steamboat and Salute, were included in the experiment. At five weeks of age, seedlings were transplanted into a 1:1 mix of infested field soil and sand. The inoculum density, determined using dilution plating as described under objective 1, was approximately 270 CFUs per gram of soil. At the conclusion of the experiment, taproot sections were taken from each plant and cultured on KM to determine if they were colonized by the pathogen.

Objective 4

Recombinant inbred lines were obtained from a cross between the cultivars Green Towers and Lolla Rossa. F_1 progeny of the cross were self-pollinated to obtain an F_2 generation. Ninety F_2 lines were transplanted into the infested field on the Davis campus that was previously used for planting date experiments. The experiment consisted of four blocks, each of which included ten plants of each line. Planting was conducted over a two day period in September (9/7 and 9/8) and plants were rated weekly for disease severity on the 1 – 4 scale described under objective 1, for six weeks. At the conclusion of the experiment, plants were rated for tap root symptoms as described under objective 1, and tap root sections were cultured on KM to document the extent of colonization by the pathogen.

Results and Discussion

Disease development at low inoculum levels

Crisphead cultivars Steamboat and Diamondback were transplanted into low inoculum plots on 4/6/2016. Based on above-ground symptoms, all plants appeared healthy at the final rating on 6/1/2016, with the exception of mild stunting in a few plants (Table 1). Based on discoloration in the taproot, mild symptoms were evident in most plants, with the average rating per block ranging from 1.06 to 1.61 for Steamboat and 1.06 to 1.22 for Diamondback (Table 1). Mean daily temperature during this experiment was 18.3 °C, with mean daily high and low temperatures of 27.0 and 10.6 °C, respectively.

Table 1. Disease severity in lettuce cultivars planted in low inoculum plots in April of 2016

Cultivar[1]	Disease severity rating[2]		Inoculum density[3]
	Above-ground	Taproot	
Steamboat	1.00 ± 0.00	1.06 ± 0.04	< 20 CFUs/gram
Steamboat	1.00 ± 0.00	1.04 ± 0.03	< 20 CFUs/gram
Steamboat	1.00 ± 0.00	1.61 ± 0.18	104 CFUs/gram
Steamboat	1.00 ± 0.00	1.05 ± 0.04	< 20 CFUs/gram
Steamboat	1.10 ± 0.10	1.38 ± 0.12	20 CFUs/gram
Steamboat	1.00 ± 0.00	1.18 ± 0.08	20 CFUs/gram
Diamondback	1.00 ± 0.00	1.06 ± 0.04	< 20 CFUs/gram
Diamondback	1.00 ± 0.00	1.08 ± 0.05	< 20 CFUs/gram
Diamondback	1.00 ± 0.00	1.12 ± 0.07	104 CFUs/gram
Diamondback	1.00 ± 0.00	1.22 ± 0.10	< 20 CFUs/gram
Diamondback	1.09 ± 0.09	1.10 ± 0.05	20 CFUs/gram
Diamondback	1.00 ± 0.00	1.11 ± 0.08	20 CFUs/gram

[1]Crisphead cultivars used in the experiments
[2]Disease severity was based on above-ground (= shoot) and below-ground (= taproot) symptoms; entries correspond to the average ± standard error
[3]Number of colony forming units of the Fusarium wilt pathogen per gram of soil

In a repeat of the above experiment, Steamboat and Diamondback were planted into the same plots on 8/16/2016. Unlike in the previous experiment, most plants showed symptoms of Fusarium wilt, and above-ground disease severity ratings were higher, ranging from 1.22 to 1.50 and 1.07 to 1.22, across all six plots for Steamboat and Diamondback, respectively (Table 2). Disease severity based on taproot symptoms was also higher than in the April planting, with mean ratings as high as 2.15 for Steamboat and 2.07 for Diamondback (Table 2). Mean daily temperature during this experiment was 21.6 °C, with mean daily high and low temperatures of 32.8 and 12.3 °C, respectively.

Table 2. Disease severity in lettuce cultivars planted in low inoculum plots in August of 2016

Cultivar[1]	Disease severity rating[2]		Inoculum density[3]
	Above-ground	Taproot	
Steamboat	1.22 ± 0.07	2.10 ± 0.06	< 20 CFUs/gram
Steamboat	1.05 ± 0.03	2.00 ± 0.13	< 20 CFUs/gram
Steamboat	1.13 ± 0.06	2.15 ± 0.15	104 CFUs/gram
Steamboat	1.25 ± 0.08	2.00 ± 0.10	< 20 CFUs/gram
Steamboat	1.50 ± 0.09	2.14 ± 0.13	20 CFUs/gram
Steamboat	1.47 ± 0.10	1.90 ± 0.10	20 CFUs/gram
Diamondback	1.08 ± 0.04	1.62 ± 0.11	< 20 CFUs/gram
Diamondback	1.10 ± 0.05	1.73 ± 0.15	< 20 CFUs/gram
Diamondback	1.20 ± 0.07	2.07 ± 0.16	104 CFUs/gram
Diamondback	1.16 ± 0.06	2.07 ± 0.17	< 20 CFUs/gram
Diamondback	1.07 ± 0.03	1.68 ± 0.15	20 CFUs/gram
Diamondback	1.22 ± 0.06	1.70 ± 0.12	20 CFUs/gram

[1]Crisphead cultivars used in the experiments
[2]Disease severity was based on above-ground (= shoot) and below-ground (= taproot) symptoms; entries correspond to the average ± standard error
[3]Number of colony forming units of the Fusarium wilt pathogen per gram of soil

Effects of amending soil with broccoli residue and chitin

Amending soil with broccoli reduced the inoculum density of the Fusarium wilt pathogen by 98% and 96% in the first and second experiments, respectively. A similar effect was apparent when chitin was included in the treatment (Figure 1).

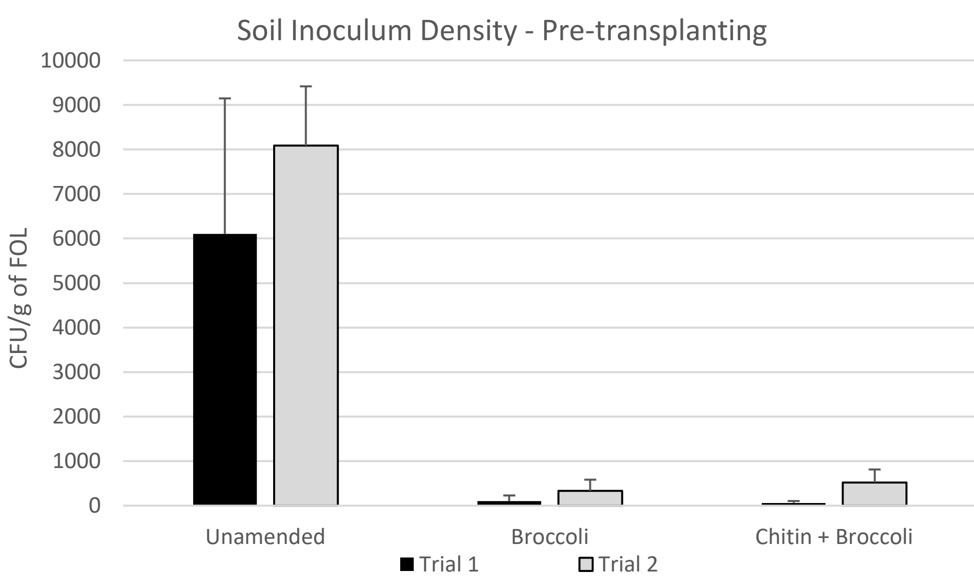

Figure 1. The inoculum density of *Fusarium oxysporum* f. sp. *lactucae* in un-amended soil and in soil to which either broccoli or broccoli plus chitin were added.

Both treatments also reduced the frequency of root infection by the pathogen (Figure 2), but the magnitude of the reduction was not proportional to the decrease in soil inoculum density. This indicates that the surviving population was still high enough for roots to encounter many pathogen propagules. This was apparent also from the extent of taproot discoloration, which did not differ between the three treatments (Figure 3).

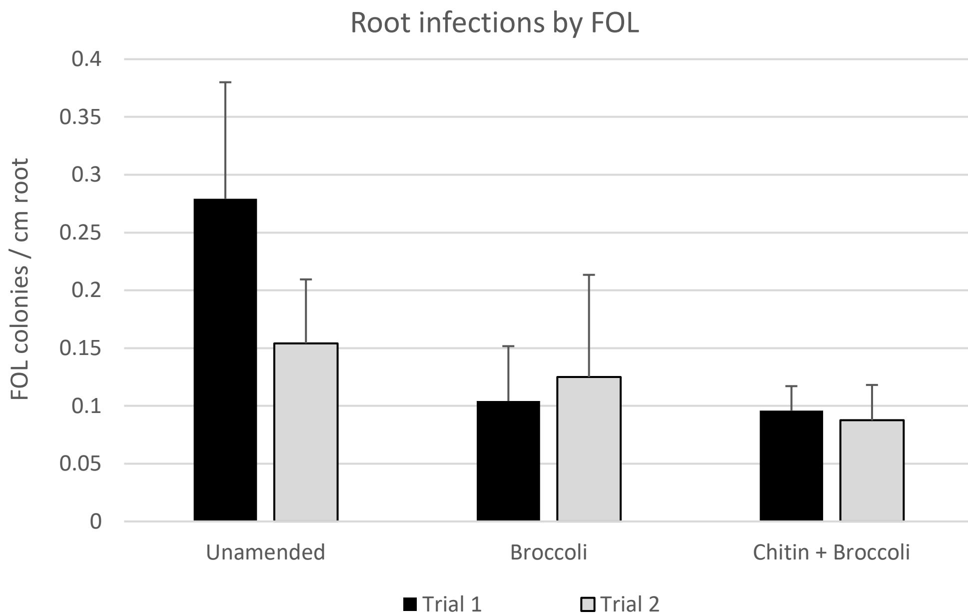

Figure 2. The number of infections per cm of root on lettuce cultivar Steamboat growing in soil that was un-amended or amended with broccoli residue or broccoli residue plus chitin.

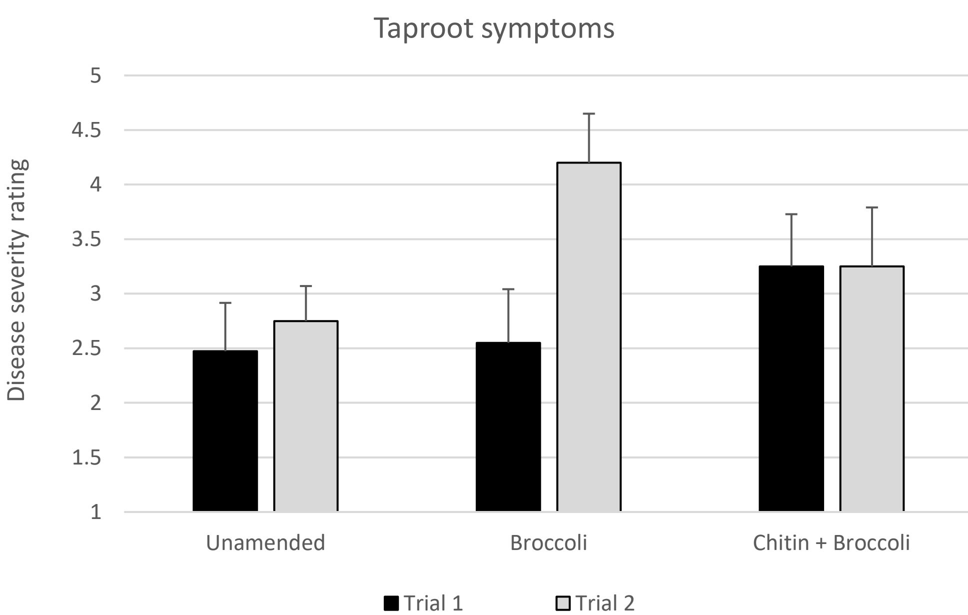

Figure 3. Mean disease severity ratings based on discoloration of the taproot of lettuce cultivar Steamboat growing in soil that was un-amended or amended with broccoli residue or broccoli residue plus chitin.

Given that enough inoculum survived to extensively colonize the taproot, it is not surprising that disease developed to a similar extent in all three treatments (data not shown), and likewise plant dry weight was similar across all three treatments (Figure 4).

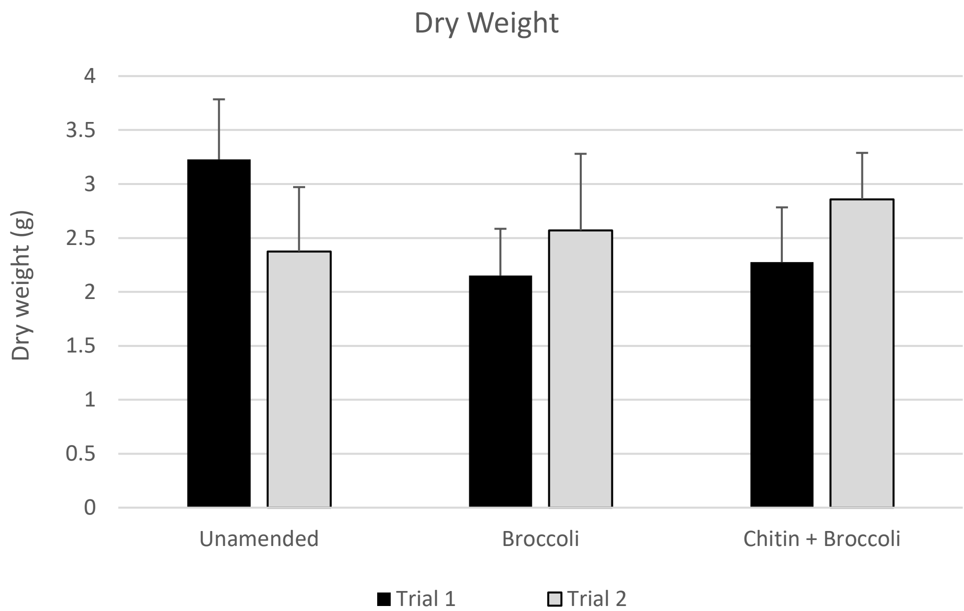

Figure 4. Mean dry weight of plants of lettuce cultivar Steamboat growing in soil that was un-amended or amended with broccoli residue or broccoli residue plus chitin.

Results of the field trial showed no beneficial effect of the chitin amendment based on either above-ground symptoms (Figure 5) or taproot symptoms (Figure 6). Results were similar for both cultivars: Bondi and Sidewinder.

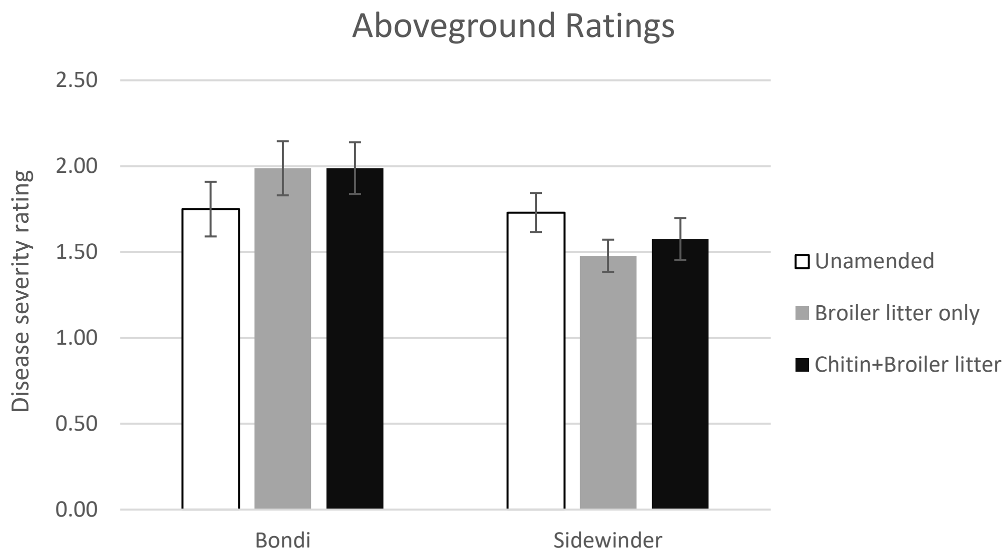

Figure 5. Disease severity in lettuce cultivars Bondi and Sidewinder growing in soil amended with broiler litter only or chitin plus broiler litter.

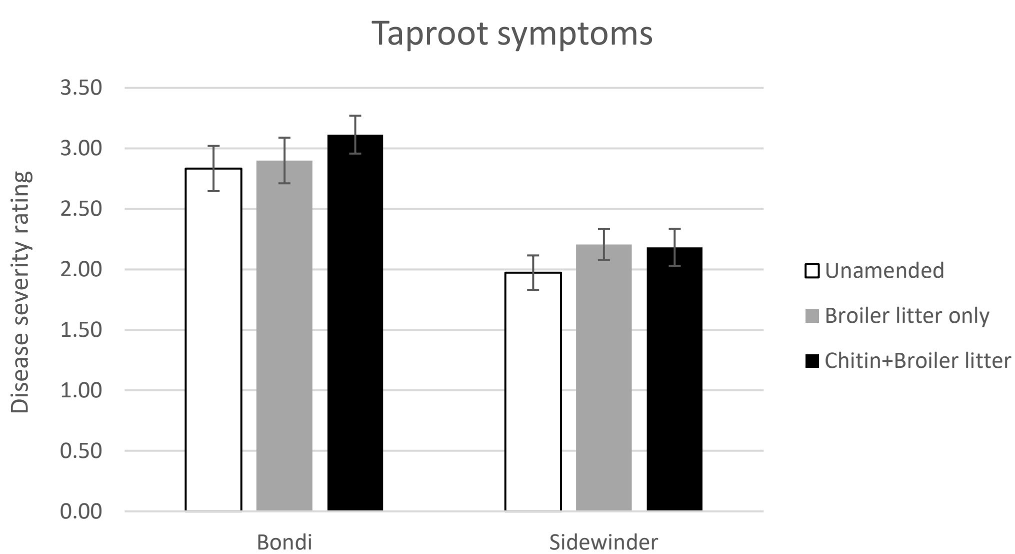

Figure 6. Disease severity based on the extent of discoloration in the taproot of lettuce cultivars Bondi and Sidewinder growing in soil amended with broiler litter only or chitin plus broiler litter.

The effect of exposure to a non-pathogenic isolate on development of Fusarium wilt

Development of symptoms was too limited to discern an effect of treatment on disease, so the assessment was based solely on the proportion of taproots colonized by the pathogen. For the cultivar Salute, 45 ± 15% of plants grown in un-amended soil had taproots infected by *F. o. lactucae*, whereas the pathogen was not detected in any plants that were previously exposed to the nonpathogenic strain. For Steamboat, exposure to the nonpathogenic isolate of *F. oxysporum* reduced the percentage of plants infected by the pathogen from 47 ± 13% to 27 ± 14%. These results suggest that pre-colonization of lettuce by a nonpathogen can enhance resistance to

Fusarium wilt. Additional experiments will be conducted to determine if this beneficial effect is detectable at higher inoculum levels.

Differences in taproot symptoms in recombinant inbred lines

Disease severity ratings based on the extent of taproot discoloration showed nearly continuous variation across the 89 lines for which data were collected (Figure 7), ranging from a low of 1.12 to a high of 2.47. The high end is relatively low, which presumably reflects mild disease pressure associated with a late planting. A subset of the 89 lines that is representative of the observed variation will be included in a test planned for 2017.

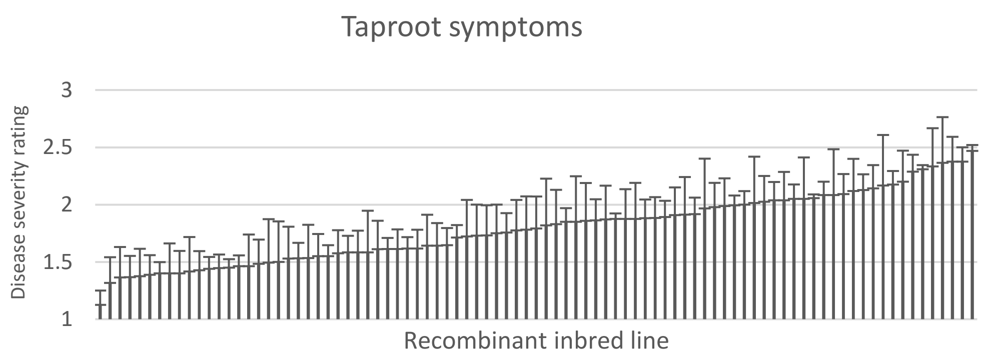

Figure 7. Disease severity in recombinant inbred lines derived from a cross between lettuce cultivars Green Towers and Lolla Rossa.

A number of results from our studies in 2016-17 have relevance to management of Fusarium wilt. First, we have documented a beneficial effect of incorporation of broccoli residue, which parallels a decline that has been reported for *Verticillium dahliae.* Although our studies did not show a decline in disease severity commensurate with the decline in inoculum, this can be attributed to the fact that our initial inoculum levels were very high. We used high levels of inoculum in order to provide better resolution of treatment effects on survival of inoculum. If we apply the observed reduction (97% on average) to inoculum levels likely to occur in a commercial field, 50 – 400 CFUs per gram of soil, the result (1.5 – 12 CFUs per gram) would likely be below the threshold for damage. We did not find any benefit of a chitin amendment alone, and combining chitin with broccoli residue did not have a greater effect than broccoli alone.

We have also demonstrated that crisphead cultivars that are susceptible to Fusarium wilt by the usual measures, can be grown without damage where inoculum levels are low and temperatures are moderate. Even with higher temperatures, impact of the disease was minimal, although the pathogen colonized the taproot in most cases, so inoculum levels in soil would increase over time. Lastly, we have confirmed what appears to be heritable variation in the extent of taproot colonization. If, as seems likely, the observed differences are determined by genes other than those associated with the resistance in romaine and leaf cultivars, it should be possible to enhance resistance by incorporating traits that reduce the ability of the pathogen to become established in roots.