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*, was first discovered in California in 1990 and has since been found in all major lettuce production areas 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. Soilborne diseases such as Fusarium wilt are most effectively controlled through the deployment of genetically resistant cultivars. High levels of resistance have been identified in romaine and leaf types but only much levels of resistance are available in crisphead cultivars. The first step in development of Fusarium wilt is infection of the root cortex. Even susceptible cultivars can sustain many of these root infections before one succeeds in reaching the core of the root, where water conducting tissue (xylem) is located. Reducing the number of root infections will therefore reduce the risk that disease will develop. Our data showed significant variation in the frequency of root infection among F_2 lines derived from a cross between a leaf and a romaine cultivar, which implies this is a heritable trait. Furthermore, there was no correlation between the frequency of root infection and disease resistance, as measured by above-ground symptoms. This implies that the two traits are independently inherited. If so, a reduction in the frequency of root infection can serve as another layer of resistance to Fusarium wilt. A nonpathogenic strain of *Fusarium oxysporum* (Fo-47) has been shown to colonize roots of tomato and induce resistance to Fusarium wilt. Under the conditions of our tests, this did not occur in lettuce. Exposure to Fo-47 in advance of the pathogen did not consistently reduce the severity of Fusarium wilt.

Objectives

1. Test recombinant inbred lines with resistance to Fusarium wilt to establish the genetic basis for differences in pathogen development in roots.

2. Establish the efficacy of a protective effect of a non-pathogenic strain of *Fusarium oxysporum* on development of Fusarium wilt.

Procedures

Objective 1

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 evaluated in a naturally infested field on the Davis campus in 2016. Based on the results of this experiment, 30 F_2 lines were selected to represent the range of variation in development of taproot symptoms. The experiment consisted of four blocks, each of which included ten plants of each line. The full experiment was conducted twice in the same field during summer of 2017. Between 20 and 24 days after planting, plants were removed for root colonization assays. One healthy appearing plant was taken from replicate plots of each F_2 included in the experiment. Roots were rinsed with water to remove soil and then were placed in a flask filled with 1% sodium hexametaphosphate to facilitate removal of clay particles on roots. Flasks were placed on a rotary shaker at 50 RPMs for 15 minutes, at which time liquid was drained from the flask and replaced with sterile water. The flask was returned to the shaker for an additional 15 minute rinse, followed by a second 15 minute rinse in sterile water. Root segments were placed on a *Fusarium*-selective medium and incubated at room temperature under continuous fluorescent light. After 5-7 days, roots were scored for the number of infections by the Fusarium wilt pathogen. A total length of 48 centimeters (cm) of roots was evaluated for each plant that was assayed.

Between 41 and 45 days after planting, all plants were rated for disease severity on a scale of 1-4, with 1 = healthy plant and 4 = a plant killed by Fusarium wilt. After ratings were completed, two plants were randomly selected from each replicate plot of each F_2. Taproots were separated from the shoot, split laterally and rated for symptoms of Fusarium wilt on a 1-5 scale, with 1 corresponding to no discoloration and 5 corresponding to an extensively rotted root. After rating, taproots were weighed and blended in 0.5% KCl and dilutions were spread over the surface of a Fusarium-selective medium. After 7-10 days under continuous fluorescent light, colonies of *F. o. lactucae* were enumerated. Data were expressed as the number of pathogen colonies per unit weight of host tissue.

Objective 2

For objective two, we used a non-pathogenic isolate of *Fusarium oxysporum* that has been shown to induce resistance to Fusarium wilt in tomato (Fo-47) and other crops. This isolate was grown on plates of potato dextrose agar, from which spores were collected and added to potting mix to obtain an inoculum density of approximately 2 million spores (= colony forming units) per gram of soil. Seed of the cultivar Steamboat was sown into potting mix and maintained in a growth chamber at day/night temperatures of 23°C/18°C. After five weeks, lettuce was transplanted into

soil amended with either 1000 (low level) or 10,000 (high level) colony forming units of the pathogen per gram of soil. The experiment was conducted twice.

Results and Discussion

The frequency of root infection varied across F_2 lines included in the first experiment (Fig. 1), ranging from 0.036 ± 0.023 colonies per cm of root to 0.151 ± 0.045 colonies per cm of root. Similar results were obtained in the second experiment (Fig. 2), in which infection frequency ranged from 0.016 ± 0.010 colonies per cm of root to 0.115 ± 0.020 colonies per cm of root. Although rankings differed between the two blocks, some lines were consistently low and others were consistently high. For example, line 66 had the lowest frequency of infection in both experiments, and 46 was near the top in both experiments.

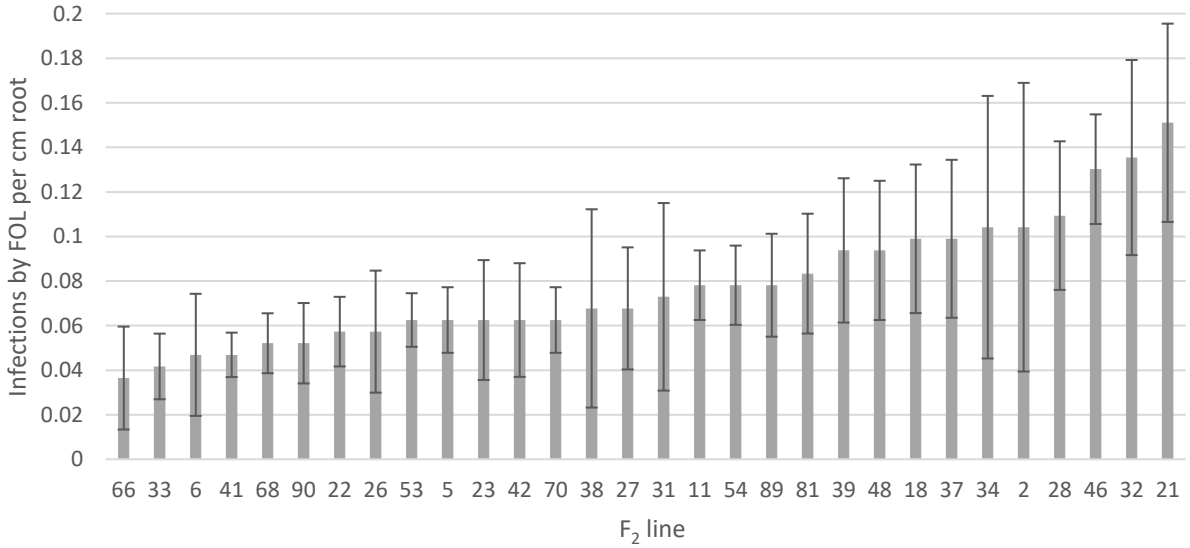

Figure 1. The frequency of infection by *Fusarium oxysporum* f. sp. *lactucae* of roots of lettuce plants representing different F_2 lines in experiment one.

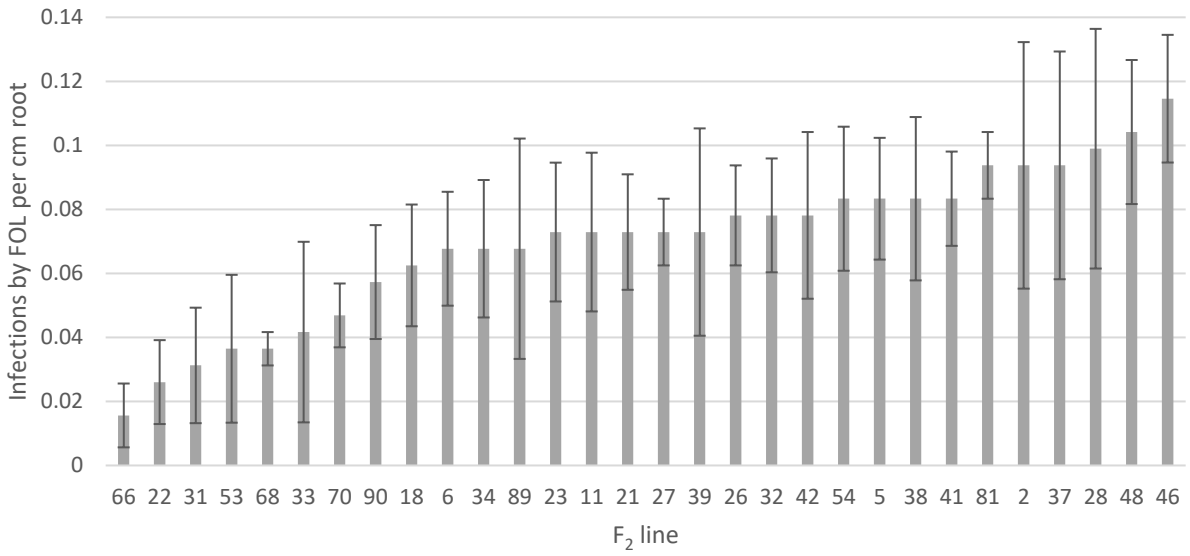

Figure 2. The frequency of infection by *Fusarium oxysporum* f. sp. *lactucae* of roots of lettuce plants representing different F_2 lines in experiment two.

Based on analysis of variance, F_2 line was a significant source of variation in ratings of disease severity based on above-ground symptoms ($P < 0.001$). The interaction between experiment and treatment was not significant ($P = 0.7258$), indicating that ranking of F_2s did not differ significantly between experiments. Averaged over both experiments, mean severity ratings ranged from 1.07 ± 0.02 to 1.94 ± 0.16 (Fig. 3).

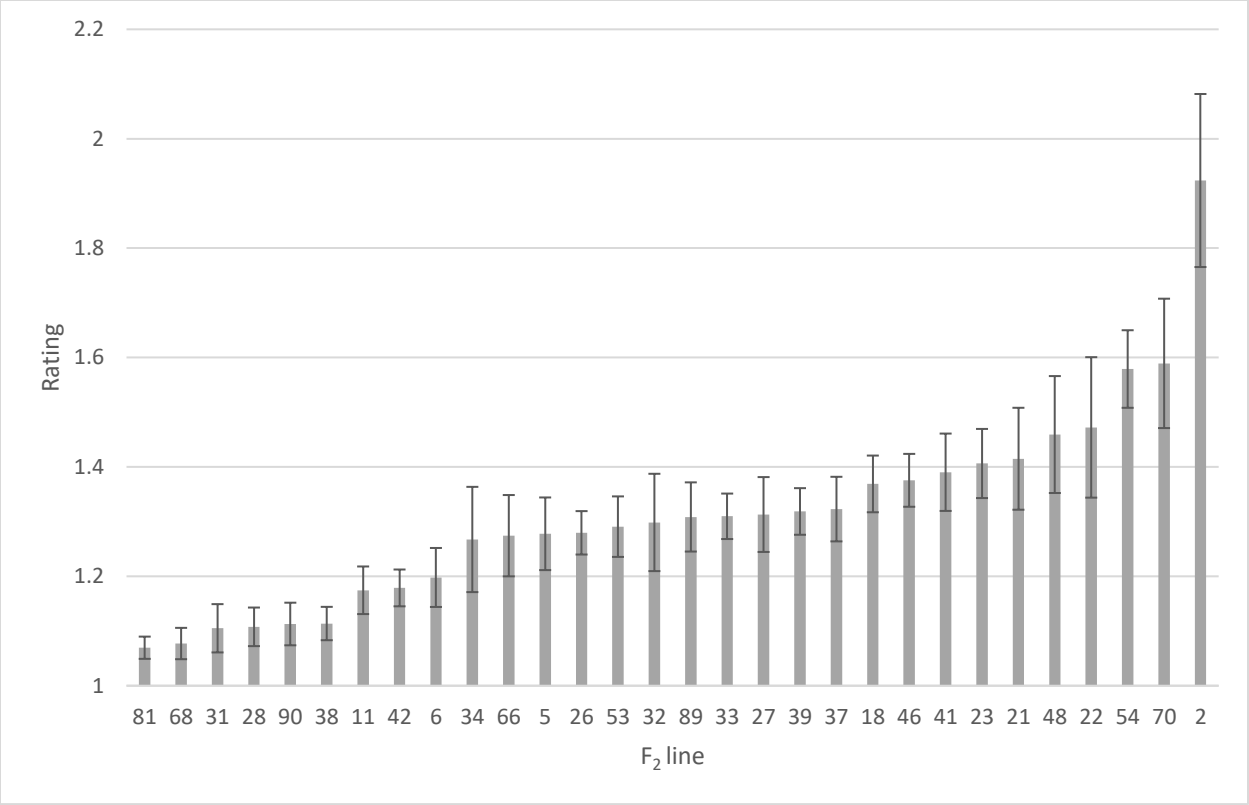

Figure 3. Severity of Fusarium wilt, based on above-ground symptoms, on F_2 lines, averaged over two experiments.

Differences in disease severity is the basis for selection of lines that are more resistant to disease. Disease symptoms are the result of growth of the pathogen in water conducting tissue (xylem). Even susceptible plants can sustain many infections of the root cortex before the pathogen reaches the xylem. Therefore, reducing the frequency with which roots become infected will reduce the risk of disease. Put another way, a lower frequency of infection will increase the level of inoculum in soil that can be tolerated without disease occurring. Our data show that there is no correlation between the frequency of root infection and resistance as we typically measure it (Fig. 4), which indicates these are independent traits. A reduction in the frequency of root infection thus represents a novel form of resistance that can serve as another layer of protection against Fusarium wilt.

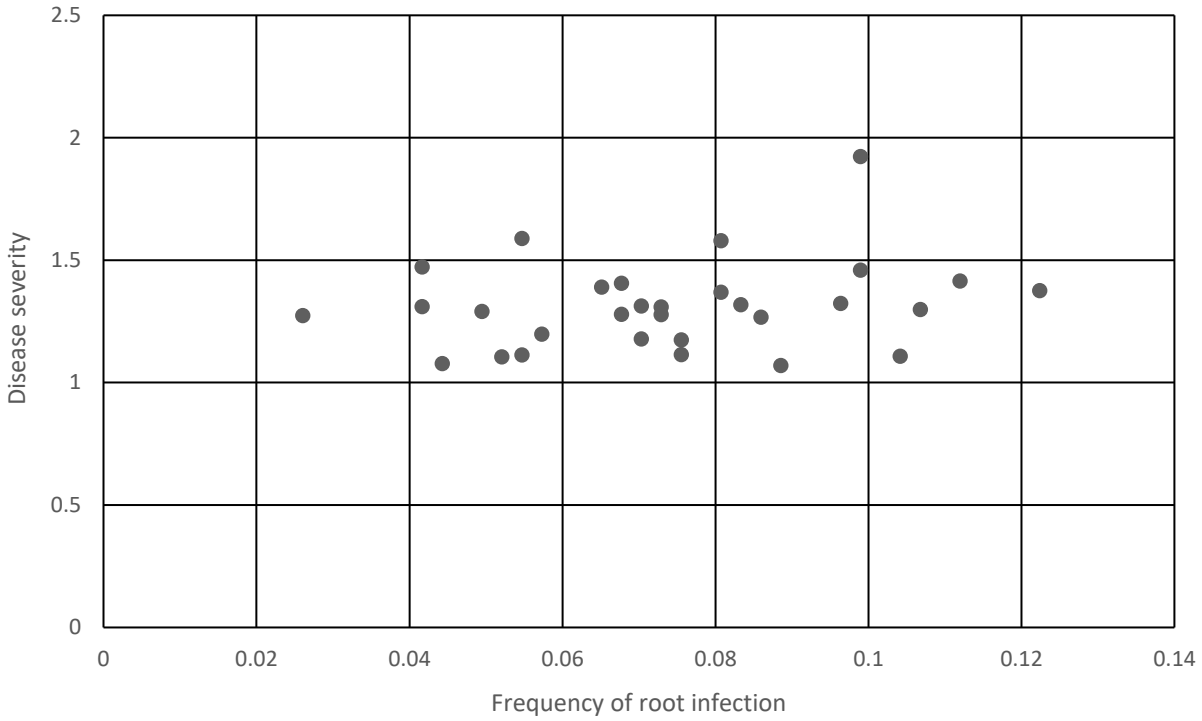

Figure 4. The relationship between severity of Fusarium wilt (based on aboveground symptoms) and the frequency with which roots become infected by *Fusarium oxysporum* f. sp. *lactucae*.

The extent to which the pathogen grew in taproot tissue was estimated by measuring the number of colony forming units per gram of host tissue. Analysis of variance showed there was not a significant interaction between experiment and treatment ($P = 0.7291$). With data pooled across experiments, F_2 lines were a significant source of variation ($P < 0.0001$). Differences in the extent of pathogen growth spanned three orders of magnitude, ranging from a low of 289 ± 183 colony forming units per gram of host tissue in F_2 line 32 to 176800 ± 34243 colony forming units per gram in F_2 line 2 (Fig. 5).

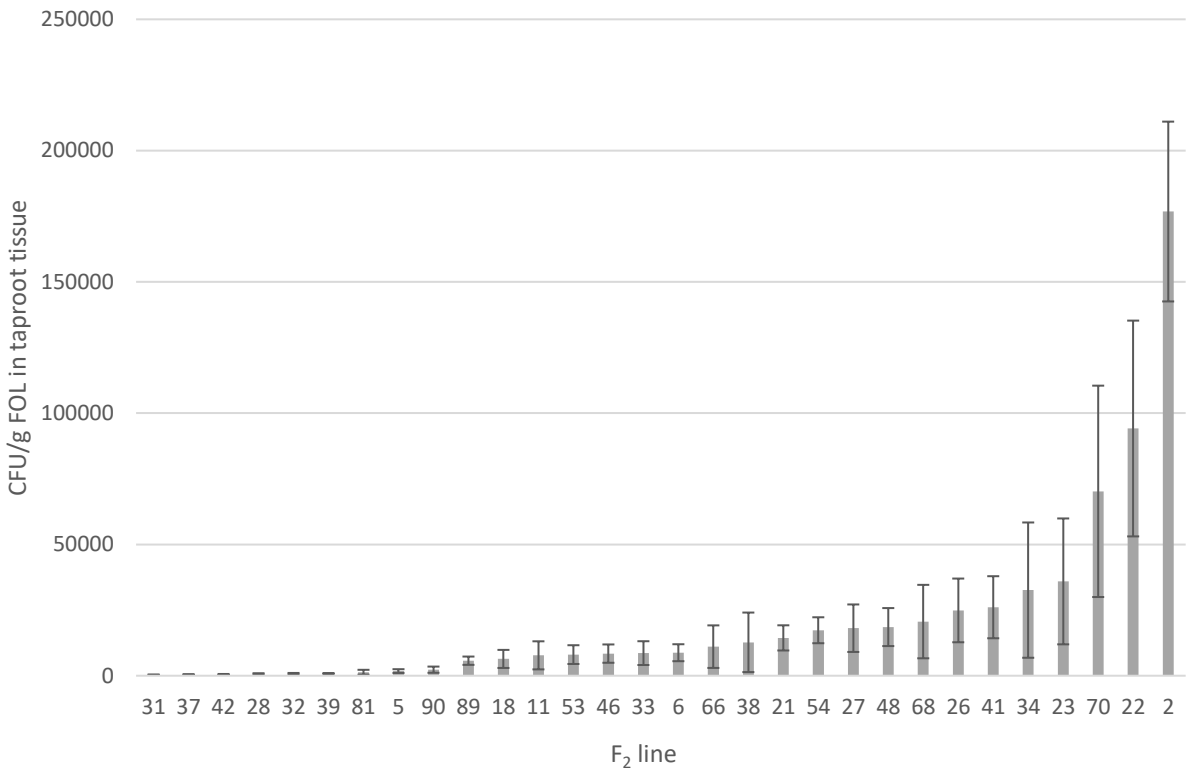

Figure 5. The extent of pathogen growth in taproots of F_2 lines, based on the number of colony forming units per gram of host tissue.

The nonpathogenic strain of *F. oxysporum* (Fo-47) has been shown to induce resistance in other crops and our experiment was designed to determine if this occurs also in lettuce. Plants exposed or not to Fo-47 were transplanted into soil infested with the Fusarium wilt pathogen. The trial was conducted twice and ANOVA showed a significant interaction between experiment and treatment ($P = 0.0170$) so data for the two trials are presented separately. In the first experiment, there was not a significant effect of prior exposure to Fo-47 at either the low or high inoculum level (Fig. 6). In the second experiment, disease severity was lower in plants that were grown in potting mix with Fo-47 but only at the low inoculum level (Fig. 7). Thus there was not a consistent beneficial effect of treatment with Fo-47 on severity of Fusarium wilt of lettuce (Fig. 8).

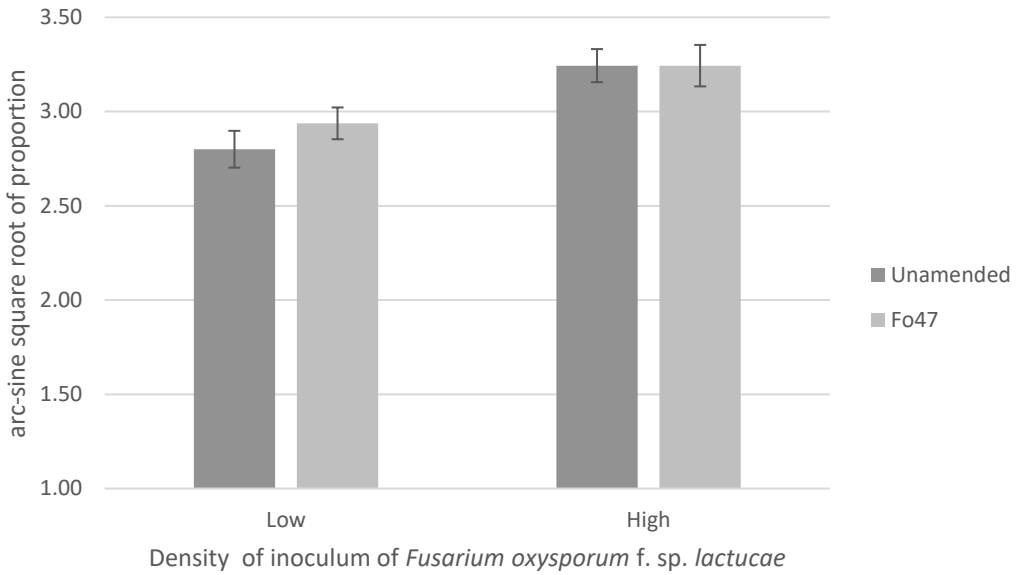

Figure 6. Severity of disease caused by *Fusarium oxysporum* f. sp. *lactucae* with and without exposure to the non-pathogenic strain, Fo-47, in trial one.

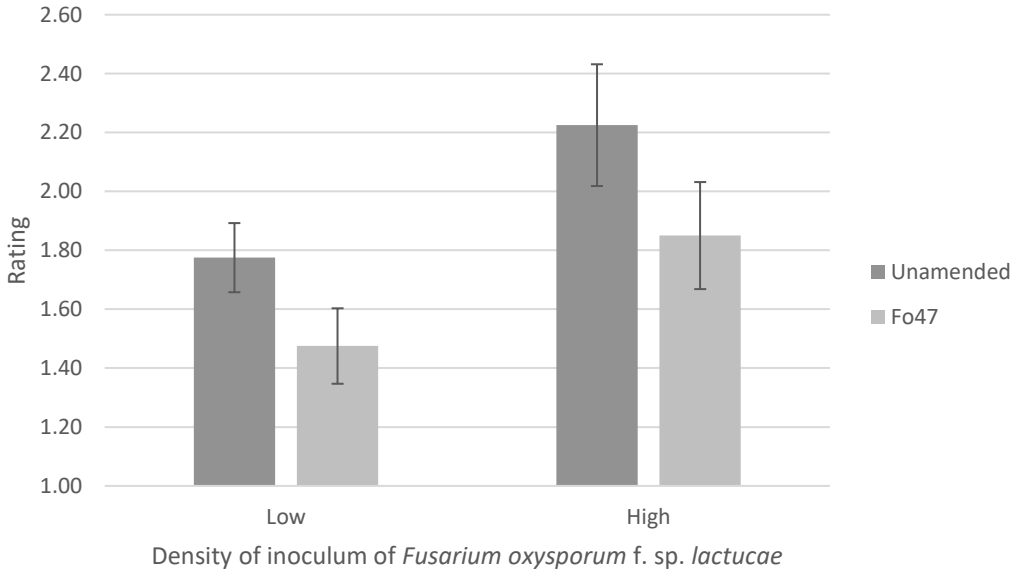

Figure 7. Severity of disease caused by *Fusarium oxysporum* f. sp. *lactucae* with and without exposure to the non-pathogenic strain, Fo-47, in trial two.