Abstract

Project title: Fusarium wilt of lettuce: management through detection, avoidance and disease resistance

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Layman's summary

Fusarium wilt of lettuce (also known as Fusarium root rot), caused by *Fusarium oxysporum* f. sp. *lactucae*, is now found in all major lettuce production areas in California and Arizona. To assess the efficacy of crop rotation as a means for reducing soilborne inoculum, we have monitored pathogen survival in fallow soil to determine the rate at which the population declines in the absence of a susceptible host. The results show a reduction of approximately 90% after one year and a slower rate of decline thereafter. The pathogen was still detectable after 34 months, at approximately 0.5% of the initial population. This result suggests that three years out of a susceptible crop may be sufficient to prevent economic damage. However, this will not be the case if a crop grown in rotation with lettuce allows the pathogen to increase. To determine if this is a potential problem, we evaluated the ability of the Fusarium wilt pathogen to colonize three crops commonly grown in rotation with lettuce: broccoli, cauliflower and spinach. All three crops were grown in naturally infested field soil and the extent of pathogen development on the tap root and feeder roots was quantified. The results showed the frequency of infection of feeder roots to be significantly lower in all three rotation crops, relative to what occurs on lettuce. Colonization of the tap root was also less extensive but recovery of the pathogen at low levels from spinach suggests this would not be the best choice for a rotation crop where Fusarium wilt is a problem. The other important component of management is disease resistance. Our previous work has shown that some romaine cultivars have higher levels of resistance than any iceberg lettuce cultivars. To better understand the basis for this difference, we tested progeny of a cross between Salinas (iceberg) and Valmaine (romaine) for their susceptibility to Fusarium wilt. The results documented segregation of resistance/susceptibility, which suggests there are good prospects for elevating the levels of resistance found in iceberg cultivars through breeding.

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Objectives

1. Continue monitoring survival of the lettuce wilt pathogen in soil

2. Determine the effect on pathogen populations of growing alternative crops in rotation with lettuce

3. Collaborate with the breeding program to identify sources of resistance to Fusarium wilt and to map the location of resistance genes

Procedures and results

<u>Objective 1</u>

In December of 2004, in collaboration with Dr. Mike Matheron at University of Arizona, we initiated a study to monitor survival of the Fusarium wilt pathogen in soil. The soil was collected from naturally-infested fields and placed in five micro-plots, which have been maintained free of vegetation. Soil samples were assayed using dilution plating and the numbers of colonies corresponding to the lettuce wilt pathogen were enumerated. The results of pathogen viability assays are shown in Figure 1. Each point corresponds to the average value for five microplots. At the first sampling date in December of 2004, average inoculum density was estimated to be 3,482 propagules per gram of soil. Viability was substantially reduced by the next sampling date in July of 2005 and has declined further since that time. As of the last sampling date in October of 2007, 17.5 colony-forming units (CFU) per gram of soil were detected. This represents less than 1% of the original inoculum level. Although it is not possible to specify a threshold inoculum level for economic damage, due to significant differences in cultivar susceptibility and the effects of temperature on symptom development, observations suggest that 17.5 CFU per gram is close to a level that would allow for production of a susceptible lettuce crop with a minimal impact of disease. Based on these findings, three years out of lettuce should be regarded as the minimum interval required for an initially high level of inoculum to drop below a threshold for economic damage. If a rotation crop supports development of the pathogen, the decline in inoculum density would be even less than what our results show.

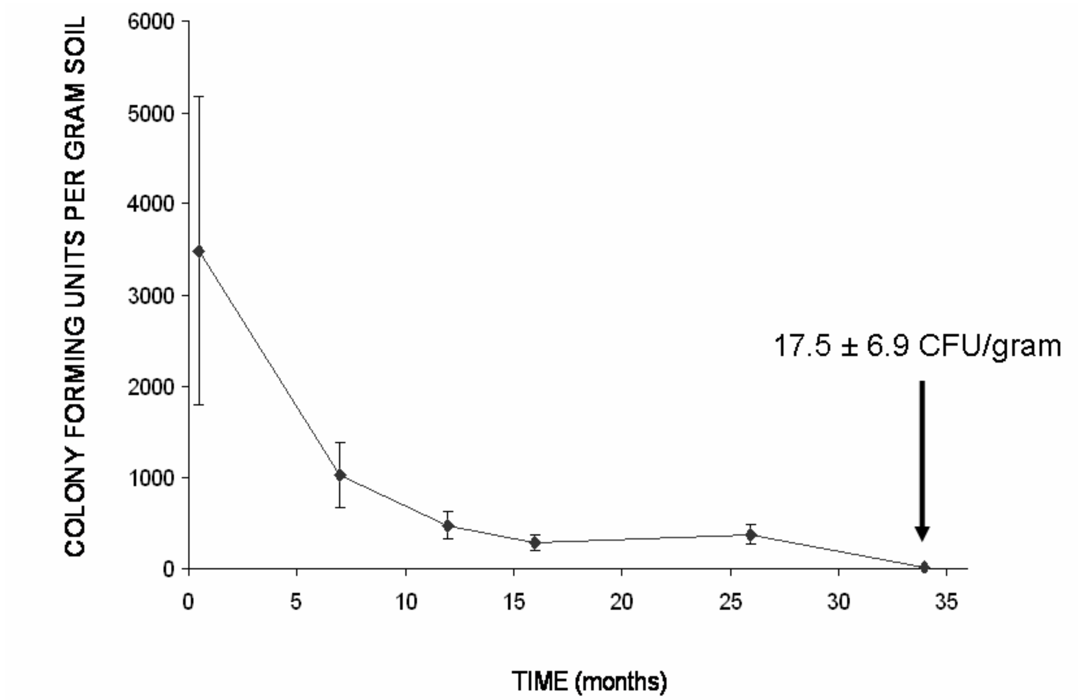

Figure 1. Each point represents the average number of colony forming units per gram of soil in five microplots. Error bars associated with each point represent 2x the standard error of the mean.

<u>Objective 2</u>

Crop rotation can result in a decline in populations of soilborne pathogens but only if they cannot extensively colonize crops used in the rotation. To estimate the magnitude of this risk for Fusarium wilt, we determined the extent of colonization of three crops commonly grown in rotation with lettuce: broccoli, cauliflower and spinach. The iceberg cultivar Salinas, and the romaine cultivar King Henry were included as controls. Each crop was seeded into five replicate plots in a naturally infested field on the Davis campus. Between 8-10 weeks after emergence, three plants were sampled from each plot. Roots were washed free of adhering soil and tap roots were separated from feeder roots. Tap roots were further subdivided into the cortex (outer layers) and the inner core of the root, which included the vascular tissue (= stele). Each set of roots: feeder, taproot cortex and stele, were weighed and blended with water for 60 seconds in a blender. Two dilution levels of each sample were spread over the surface of three Petri plates containing Komada's selective medium (KM). Plates were incubated at room temperature under 24 hours of fluorescent lighting for 5-7 days, after which colonies of *F. o.* f. sp. *lactucae* and other *Fusarium* spp. were enumerated. In addition, a subset of the feeder roots was placed intact on plates of KM. These were incubated as described above and the number of pathogen colonies per centimeter of root was determined.

Colonization of feeder roots ranged from 3.7 ± 2.2 colonies/meter (spinach) to 148 ± 12.7 colonies/meter (Salinas) (Figure 2). Analysis of variance indicated that the effect of crop on colonization of feeder roots by *F. o.* f. sp. *lactucae* was significant ($P < 0.001$). Colonization of feeder roots by other *Fusarium* spp. ranged from 35 ± 4.3 CFU/meter (spinach) to 68 ± 9.6 CFU/meter (broccoli), but these differences were not significant ($P = 0.24$). These results are consistent with expectations that only the specialized pathogen, *F. o*. f. sp. *lactucae*, discriminates between lettuce and the non-host crops.

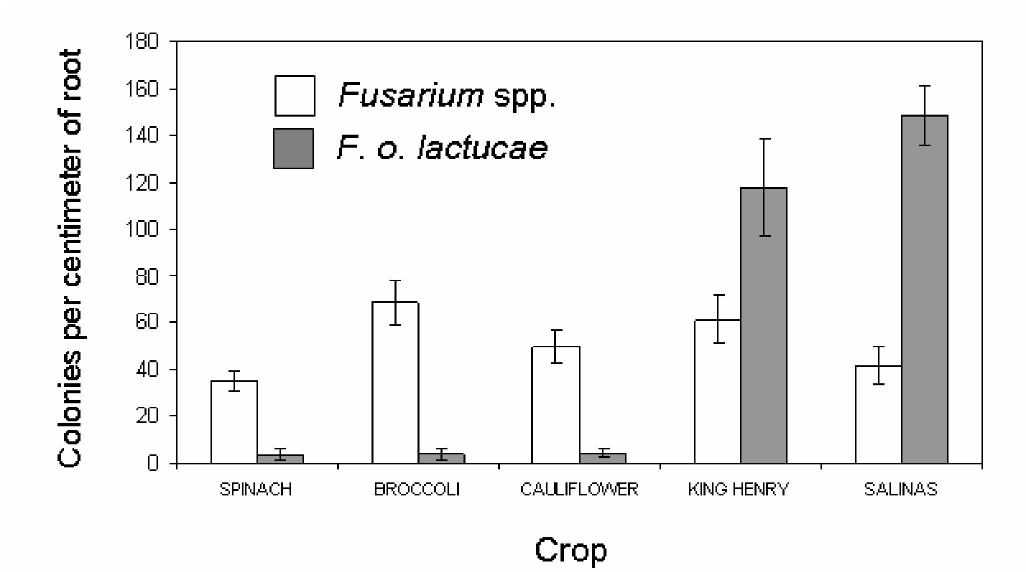

Figure 2. The height of each bar represents the number of colonies per unit length of feeder root. Error bars correspond to 2x the standard error of the mean.

These results suggest that broccoli, cauliflower and spinach are infected at a significantly lower frequency than lettuce, whether resistant (King Henry) or relatively susceptible (Salinas). However, these data do not reveal the extent to which the pathogen may develop on infected roots, something that is more apparent from tap root colonization data described below. Also, differences in infection frequency between the highly resistant cultivar, King Henry, and the more susceptible Salinas are quite modest. Thus, differences in the susceptibilities of these two cultivars must reflect mechanisms of host defense that affect post-infection development of the pathogen.

Insight into the extent to which a pathogen can build-up on a rotation crop may be gleaned from estimates of pathogen biomass in colonized plants. Such estimates were obtained by determining the number of pathogen colonies that developed from homogenates of plant roots, as shown in Figure 3 below. Colonization of the cortex by *F. o.* f. sp. *lactucae* ranged from 14 ± 2.6 CFU/gram (cauliflower) to 13,000 ± 1,800 CFU/gram (Salinas). Although Salinas was more extensively colonized than the more resistant cultivar King Henry, this difference was not statistically significantly ($P = 0.85$). Additionally, the level of colonization detected in the cortex of cauliflower was not significantly different than that of either broccoli or spinach ($P = 0.51$ and 0.32). Colonization of the cortex of lettuce (Salinas and King Henry) was significantly greater than what occurred in the other crops ($P < 0.001$). Colonization of the interior portions of the root (stele) ranged from 0 CFU/gram (cauliflower and broccoli) to 14,000 ± 5,300 CFU/gram in Salinas. The lettuce wilt pathogen was recovered from the stele of spinach at levels (139 ± 32 CFU/gram) ten-100 times lower than for lettuce (Salinas and King Henry). Although this constitutes a very low level of colonization, it may have a significant effect on the pathogen population where spinach is grown in rotation with lettuce. Consequently, in fields where Fusarium wilt has been a problem on lettuce, spinach may not be the best choice for a rotation crop.

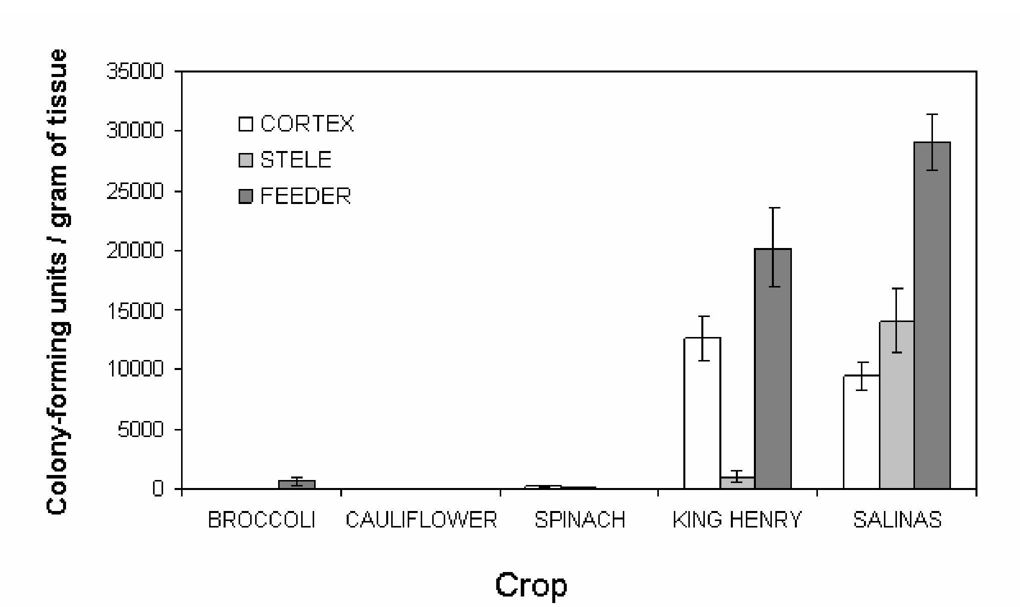

Figure 3. The height of each bar represents the number of colony-forming units per gram fresh weight of plant tissue. Error bars correspond to 2x the standard error of the mean.

Objective 3

To better understand the genetic basis for resistance to Fusarium wilt, we have examined the relative susceptibilities of inbred lines (families) derived from a cross between Salinas and Valmaine. A total of sixty eight families, plus the two parent cultivars were established in two replicate blocks in an infested field on the UC Davis campus in August of 2007. Beginning in early September, plants were rated at weekly intervals until a final rating was taken on 9 October. As of that date there was little or no change in the rating for any of the entries, relative to the previous rating one week earlier. Based on the final rating, the families displayed nearly continuous variation in susceptibility to Fusarium wilt, with some showing little or no damage (the lowest rating was 0.03 on a 0-3 scale), others being completely susceptible and the rest showing intermediate reactions to the disease (Figure 4). Ratings for the same family in different blocks were generally quite similar and the overall correlation was strong (R^2 = 0.704). The results of this experiment document segregation of the determinants of resistance to Fusarium wilt. Thus it should be possible to transfer the higher levels of resistance found in some romaine cultivars into iceberg cultivars.

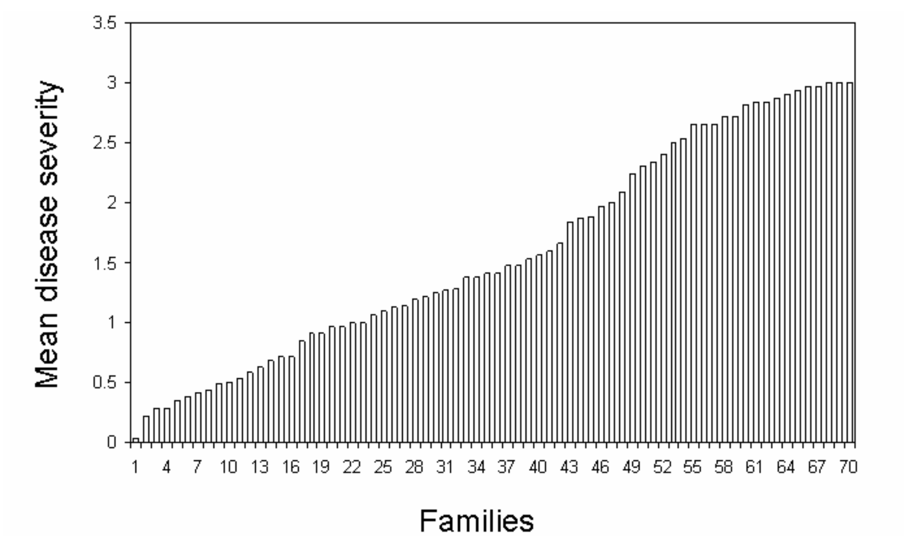

Figure 4. The height of each bar represents the average disease severity rating for 68 families and both parents of the cross (Salinas and Valmaine).