

## **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) is caused by the soilborne fungus *Fusarium oxysporum* f. sp. *lactucae*. One component of Management of this disease is crop rotation, which can help to reduce soil inoculum levels. However, this benefit will only be realized if rotation crops do not support development of the pathogen to a significant extent. For this reason, we have examined three cool season crops that might be grown in rotation with lettuce: broccoli, cauliflower and spinach. The results of our experiments over the last two years showed that all three crops sustain root infections by the lettuce wilt pathogen but that development within the root is quite limited. However, spinach appears more conducive to growth of the pathogen than either broccoli or cauliflower and thus should probably not be grown in rotation with lettuce where Fusarium wilt has been a problem. The other critical component of disease management is genetic resistance, which will be increasingly important as Fusarium wilt becomes more widespread. For this reason, we have worked with the Michelmore lab to examine the inheritance of resistance and to lay the foundation for obtaining elevated levels of resistance in iceberg cultivars. To this end we have examined the relative susceptibilities of inbred lines derived from a cross between the resistant romaine cultivar 'Valmaine' and the moderately resistant iceberg cultivar 'Salinas'. The results indicate that many of the inbred lines have resistance comparable to that found in the resistant parent (Valmaine) and some of these have the general appearance of iceberg cultivars. Thus it should be possible to move the determinants of resistance from Valmaine into the genetic background of a commercially acceptable iceberg type.

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## **Objectives**

1. Determine the potential for crops grown in rotation with lettuce to support development of the lettuce wilt pathogen
2. Collaborate with the breeding program to identify sources of resistance and combine resistances from different sources

## **Procedures**

### Objective 1

One component of management of wilt diseases is crop rotation, which can result in a decline in pathogen populations. This can be effective provided a pathogen does not extensively colonize the non-host crops. We have evaluated the magnitude of this risk for Fusarium wilt, by quantifying the extent of colonization of three crops commonly grown in rotation with lettuce: broccoli, cauliflower and spinach. Our experiments also included the iceberg cultivars 'Early Queen' (susceptible) and 'Salinas' (moderately resistant), and the romaine cultivar 'King Henry' (resistant). The experiment conducted in 2008 was essentially a repeat of a similar experiment conducted in 2007. Each of six crops 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. Feeder roots were placed directly onto Petri plates containing Komada's selective medium. 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* were enumerated. Tap roots were subdivided into the cortex (outer layers) and the inner core of the root, which included the vascular tissue (= stele). The taproot cortex and the stele were separately weighed and blended with water for 60

seconds in a blender. Two dilution levels of each sample were spread over the surface of Petri plates containing Komada's selective medium (KM). These were incubated as described above and the numbers of pathogen colonies per centimeter of root were counted.

## Objective 2

To better understand the genetic basis for resistance to Fusarium wilt, we have examined the relative susceptibilities of recombinant inbred lines (RILs) derived from a cross between Salinas and 'Valmaine'. This study was initiated in 2007, when sixty eight families, plus the two parent cultivars were established in two replicate blocks in an infested field on the UC Davis campus. The same location was used for a similar experiment in 2008. This experiment included 89 families and both parents: Salinas and Valmaine. Transplants were established in June and were rated at weekly intervals thereafter until mid-August.

## **Results and Discussion**

Colonization of feeder roots ranged from  $3.0 \pm 0.8$  colonies/meter (spinach) to  $85.8 \pm 17.1$  colonies/meter (King Henry) (Figure 1). Analysis of variance indicated that the effect of crop on colonization of feeder roots by *F. o. f. sp. lactucae* was significant ( $P < 0.001$ ). These results are consistent with findings from 2007, and show that non-host crops are infected much less frequently than lettuce. However, there was little difference in frequency with which lettuce cultivars were infected, even though they differ in susceptibility. It is apparent therefore that that resistance is not expressed at this level and must be associated with subsequent development of the colonies established in the root cortex.

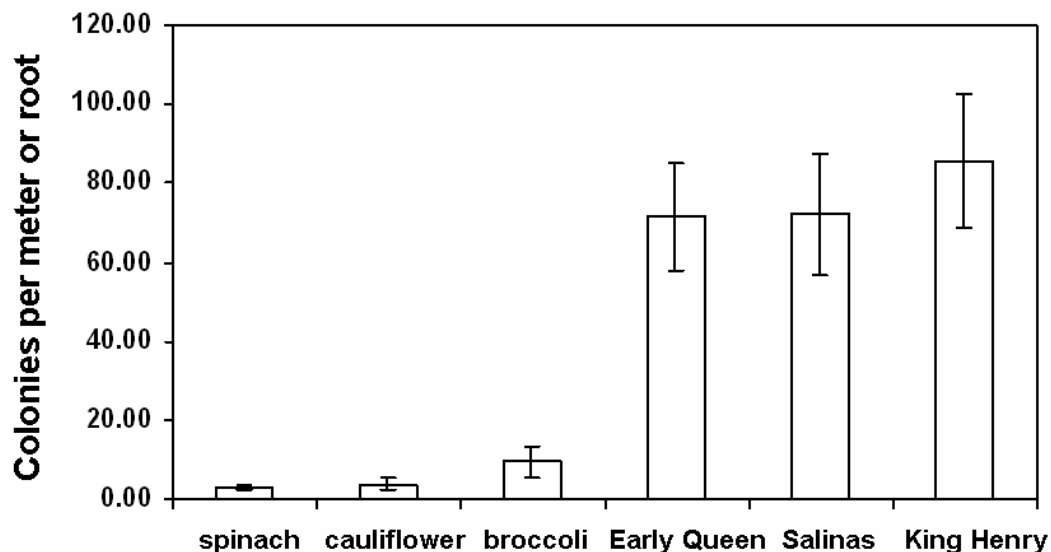


Figure 1. The height of each bar represents the number of colonies per unit length of feeder root. Error bars correspond to 2x the standard error of the mean.

Differences in the extent to which pathogen colonies develop can be estimated as the amount of pathogen biomass in colonized plants. Such estimates were obtained by determining the number of pathogen colonies that developed from homogenates of the root cortex (Figure 2). The most extensive development occurred in the susceptible cultivar Early Queen, with over 800 colony-forming units per gram of root cortex. Much lower levels were found in King Henry, with Salinas being intermediate. Thus, although all three cultivars are infected at nearly the same frequency, the extent to which the pathogen develops in the cortex is proportional to their susceptibility. Rotation crops supported limited or no detectable growth of the pathogen in the root cortex (Figure 2). Although not apparent from Figure 2, because of the scale used to present the data, the pathogen was recovered at a very low level from broccoli: 8.5 colony-forming units per gram; it was below the detection threshold in cauliflower.

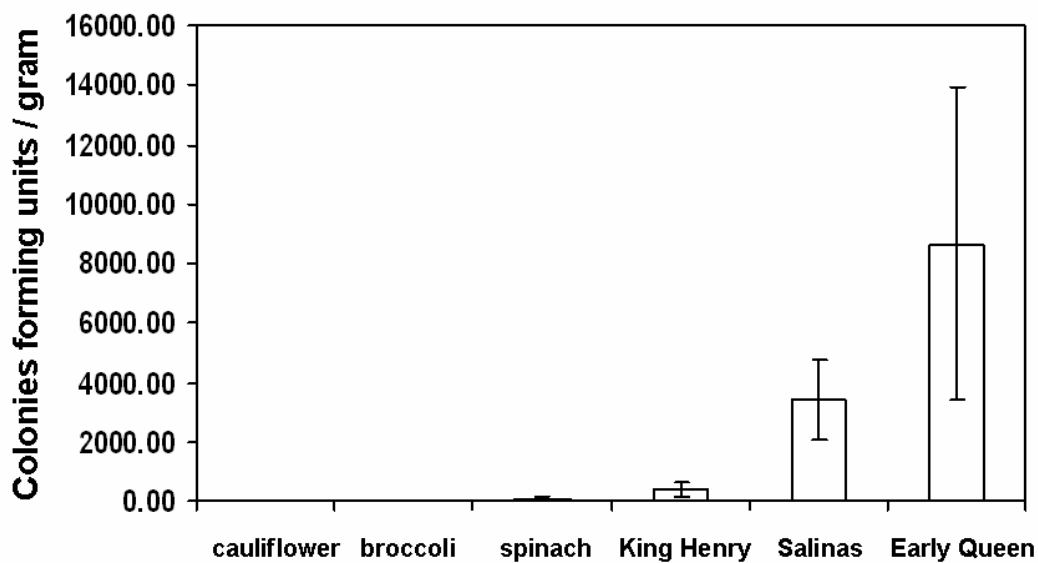


Figure 2. The height of each bar represents the number of colony-forming units per gram fresh weight of root cortex. Error bars correspond to 2x the standard error of the mean.

A further indication of the capacity for a crop to sustain populations of the pathogen is revealed by the pathogen's development in the vascular stele. As shown in Figure 3 below, the pattern is similar to what was observed for the root cortex, with the highest levels found in Early Queen, lower levels in Salinas and barely detectable levels in King Henry (116 colony-forming units per gram). The pathogen was not detectable in either broccoli or cauliflower but was present at a very low level in spinach (37 colony-forming units per gram). Similar results were obtained in 2007. Although colonization of spinach appears relatively modest, on a commercial field scale it could have a significant impact on the population of the pathogen. That is, it could allow the pathogen to produce propagules that will compensate to some degree for those lost by attrition in the absence of a susceptible lettuce crop. For this reason, in fields where *Fusarium* wilt has been a problem on lettuce, spinach would not be the best choice for a rotation crop.

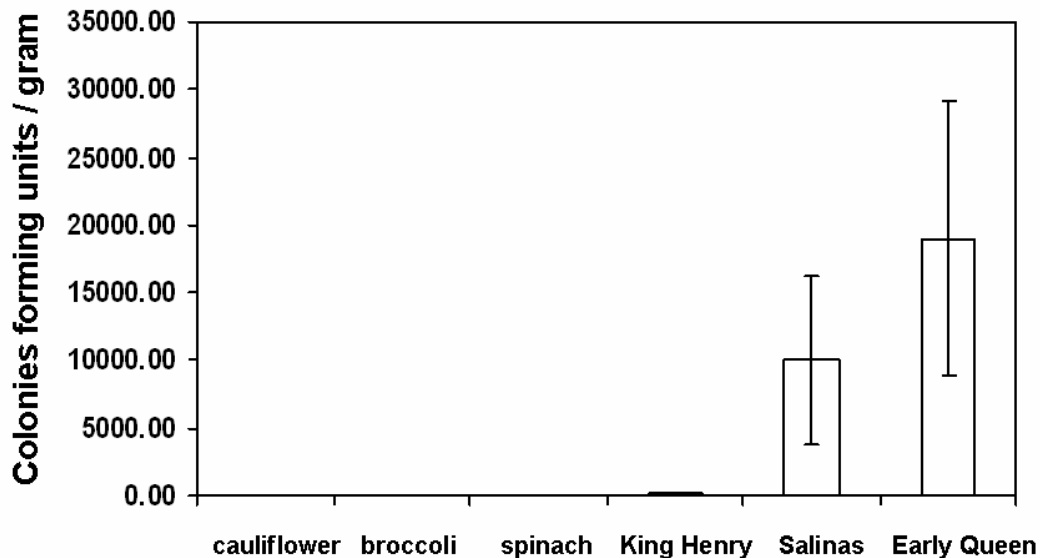


Figure 3. The height of each bar represents the number of colony-forming units per gram fresh weight of the vascular stele. Error bars correspond to 2x the standard error of the mean.

In the field test of RILs obtained from a cross between Salinas and Valmaine, clear differences were apparent between resistant and susceptible entries by four weeks after planting (Figure 4). 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 0.025 on a 0-3 scale with 0 corresponding to healthy and 3 to a dead plant. Other entries were completely susceptible and the rest showed intermediate reactions to the disease (Figure 5). Valmaine and Salinas had final disease ratings of 0 and 1.3, respectively.

The results of our experiments (2007 and 2008) have documented segregation for resistance in RILs derived from this cross, which indicates that the two parents have different determinants of resistance, at least in part. Analysis conducted by Michelmore's group suggests that two genes affecting resistance may be segregating in this population. A number of RILs were highly resistant and some also had the general appearance of iceberg cultivars, suggesting they are not far from an acceptable commercial type. After the final rating, plants were allowed to flower and crosses were made to further the process of introgressing resistance from Valmaine into iceberg types. This material will assist breeders interested in developing cultivars with higher levels of resistance to *Fusarium* wilt.



Figure 4. Composite photograph showing differences in susceptibility to Fusarium wilt four weeks after transplants were established in an infested field on the Davis campus.

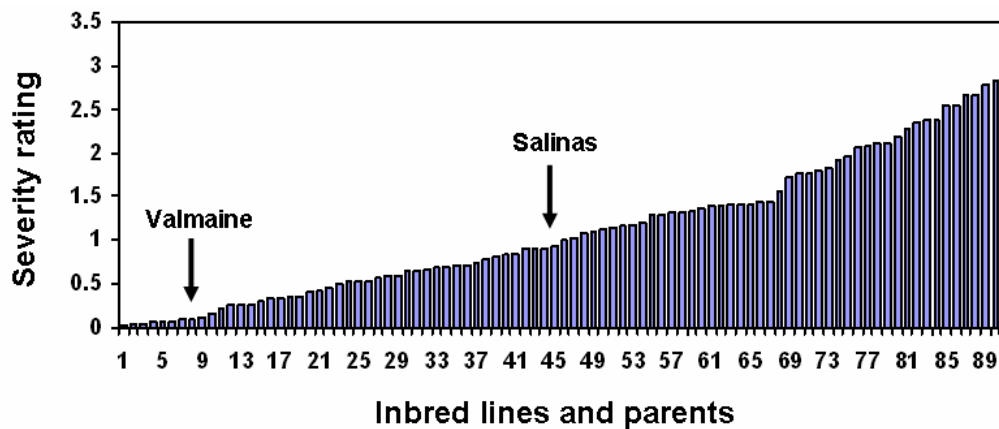


Figure 5. The height of each bar represents the disease rating for each entry. Ratings for parent cultivars are indicated by arrows.