Arizona Iceberg Lettuce Research Council
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Project title: Variation in virulence among Yuma isolates of *Fusarium oxysporum* f.sp. *lactucae*

Principal Investigators: Barry Pryor, Professor, Department of Plant Pathology, University of Arizona, Tucson

Introduction: The wilt diseases of many crops caused by the pathogenic fungus *Fusarium oxysporum* encompass complex interactions between host genetic diversity and fungus genetic diversity. There are strains of the fungus that only infect certain crops, and each is known as a "special form", or in Latin "forme specialis" (f.sp.). The forme specialis that infects lettuce is known as *Fusarium oxysporum* f.sp. l*actucae* (Fol), and is responsible for the devastating disease known as Fusarium wilt of lettuce.

Within each forme specialis, there is also addition genetic diversity and this is revealed in "races". Each race can infect certain cultivars of that host and not others, and across the many different forme specialis of *Fusarium*, whether they infect cotton, pepper, bananas, tobacco, etc, there are well known established races with differential pathogenicity on specific cultivars of the host plant. For Fol, there are 3 established races, race 1, 2, and 3. Most of the strains that have been spread around the world, including into California and Arizona, are race 1, which is fortunate from a management perspective in that growers can predict which cultivars might be most susceptible and which might be more tolerant. Unfortunately, new races can periodically emerge, and an Fol race 4 has recently been reported in several European countries.

Now there is a third level of genetic variation with pathogenic *Fusarium* strains, including Fol, and this is variation in virulence within each race. Not all isolates within a race are equal; some are weakly pathogenic and some are strongly pathogenic. This variation in virulence has not be well reported in literature, but it is noted in the field. Some fields that are infested with Fol have serious disease problems and some fields not as much. And the symptoms on the plant can be different as well, with some plants showing slight vascular discoloration and other plants showing serious and extensive necrosis of the entire core of the lettuce root.

Now we know that there is genetic variation within races of *Fusarium* that are pathogenic on many crops, and this has been well published research using vegetative compatibility tests. And ongoing studies are looking at this level of variation in Fol as well. However, little work has been done to examine the variation in virulence within Fol, and most importantly, within race 1 of Fol found in the Yuma lettuce production area. Understanding this variation in virulence may help explain some of the differential expression in disease seen in this region, and assist in developing better tools for managing this disease. Very importantly, understanding the variation in virulence will be critical as new lettuce cultivars are introduced into this area for improved disease management.

Previous research conducted on Fol at the University of Arizona has focused on developing rapid methods for detecting Fol infection in lettuce as soon as possible, with high sensitivity and

specificity, using DNA-based detection methods. These studies have utilized greenhouse trials that artificially infect lettuce seedlings grown in pots. The value of greenhouse studies is that we can infect lettuce with different inoculation levels, simulating different field infestation levels, and do this for crops in rapid successtion. There is no need to wait for a lettuce planting period as we can grow lettuce in the greenhouse year round. And in these studies, we can use whichever strains of Fol are handy and whichever susceptible cultivar of lettuce are readily available.

While these studies have been valuable in providing us a platform for exploring the infection process and the rate at which disease develops in a lettuce plant, over the course of this research we also discovered some interesting aspects of virulence that we never saw before. First, not all isolates of Fol race 1, collected in the Yuma area, exhibit the same level of virulence. In other words, some isolates cause disease very quickly, some more slowly, and some hardly at all. Second, not all isolates of Fol race 1, collected in the Yuma area, have the same range of pathogenicity across lettuce cultivars tested. In other words, some isolates will infect all cultivars tested whereas others may fail to infect some specific cultivars. Now this is unusual because all of the isolates of Fol are race 1, and have been genetically tested as race 1 by standard DNA-based methods. And yet, here we still see differential pathogenicity, even within a defined race.

And third, in the course of developing suitable greenhouse-based methods for our DNA-based work, we have had to explore the use of different potting soil mixtures to find the right one for lettuce in the greenhouse that is appropriate for all seasons. To date we have evaluated 5 different mixtures, including some that have field soil incorporated into them. What we have seen that is surprising is that Fol isolates perform differently against different lettuce cultivars depending on what soil mix is being used. Now this is very unusual and suggests that the soil environment has an impact on whether or not a particular isolate of Fol will cause disease against a particular cultivar of lettuce. The cause of this interaction is not known, and could be related to the microbiology of the soil mix, the pH of the soil mix, the water-holding capacity of the soil mix, etc. However, understanding this interaction between soil, the pathogen, and the cultivar will certainly have profound near-term and long-range impacts in our lettuce breeding programs and our management of soil health. More research is clearly needed in this area to improve management of Fusarium wilt of lettuce. This is the purpose of this research.

Materials and Methods.

The principal goals for this project were to conduct a series of trials to test the virulence of select Fol isolates against select head lettuce cultivars planted in different soil types under greenhouse conditions. We understand that there may be other environmental factors that also contribute to the expression of disease, for example, inoculation amount. We have consider these factors and other in subsequent studies that follow these efforts.

Activity 1: Assemble strains of FOL

Two sampling trips were taken to the Yuma production area. In each trip, 8 fields were sampled. For some fields, soil was collected and brought back to the lab. In other fields, infected lettuce heads were returned to the lab. For each field, location and sample date were recorded. From

each sample, whether soil or lettuce, standard fungus isolation techniques were used to recover the target FOL isolates

Activity 2. Collect a selection of cultivars to be tested.
20 cultivars of head lettuce were initially selected for initial screening for suitability in pathogenicity tests. Ultimately 10 cultivars were selected based upon known Fol susceptibility and robust scores from previous field-based disease susceptibility trials and consistent performance in preliminary greenhouse tests.

Activity 3. Soil was collected from 2 uncontaminated fields in Arizona, 1 in Yuma and 1 east of Wellton along the Gila River Valley. The soils will be confirmed uncontaminated with Fol by soil plating techniques and by testing against lettuce seedlings in the greenhouse.

Activity 4. Test FOL strains against lettuce cultivar or accession.
Perform a series of 4-week trials in which 10 specific Fol strains are tested against 10 specific lettuce cultivars. Additional trials examined the effect of soil type in disease expression and on inoculation method.

Six greenhouse trials were completed. Each trial was set up as a 3-factorial design with 3 blocks for statistical robustness. In each trial, the first factor was soil. There were three soil types used, one standard greenhouse potting mix and 2 mixes of field soil/vermiculite (added for soil porosity and water retention) using the two soils from different fields. This factor was present in all trials.

For each for each trial 3-4 different strains of Fol were tested against a set of 10 lettuce cultivars. All cultivars were selected based up established field ratings obtained in previous studies. For each strain/cultivar combination, there were 3 test pots (1 gal pots) per block and scores for the 3 pots were averaged to obtain a single combination score for that block. Scores for all blocks were analyzed by ANOVA as a 3-factorial randomized complete block design.

Each trial was repeated (2X), followed by the next trial of 3 different Fol isolates against the same 10 lettuce cultivars. A total of 10 Fol isolates were tested. Scoring was performed 7, 14, and 21 days post inoculation. Scoring was two-fold: plant height measured in cm, and plant disease severity measured on a 5-point rating scale. 1= no disease, 2= wilt of one or two leaves, 3= wilt of all leaves, 4= wilt of all leaves and necrosis, 5= plant dead. Chlorosis was noted but not scored unless it becomes prevalent.

Results.
Activity 1: Assemble strains of FOL
From the two sampling trips that were taken to the Yuma production area, 16 fields were sampled. From either soil or lettuce, one to several isolates were recovered per field. All recovered isolates were tested genetic identity and for pathogenicity. From these original isolates, 10 were selected for further study. Among these 10 isolates, variation in virulence exhibited during preliminary screens was noted. For each field, location and sample date were recorded. See Table 1.

Activity 2. Collect a selection of cultivars to be tested.

From a review of previous work by Matheron et al, 10 cultivars were selected based upon robust scores from one to several previous field-based disease susceptibility trials and consistent performance in preliminary greenhouse tests. The assembled set of 10 cultivars included isolates that exhibited high tolerance to the pathogen, those that exhibited no tolerance to the pathogen, and some that were intermediate. See Table 1.

Activity 3. Soil was collected from 2 uncontaminated fields in Arizona, 1 near Yuma and 1 east of Wellton along the Gila River Valley. The soils were tested for FOL infestation by soil plating techniques and by testing against lettuce seedlings in the greenhouse, and were found negative by both techniques. These soils were used in subsequent greenhouse tests for pathogenicity.

Activity 4. Test FOL strains against lettuce cultivar or accession.
Trials were conducted testing specific strains of Fusarium against specific cultivars of lettuce using three different soil types. Additional trials examined the effect of inoculation method on disease expression. The most immediate distinction in pathogenicity results was evident between greenhouse potting soil and field soil. Whereas greenhouse potting soil promoted lettuce plant growth and development, it did not promote disease development and thus, did not provide a range of responses between host (lettuce) and pathogen (Fusarium) that field soil provided (data not shown). Differences between field soil types (Yuma field 1 vs Wellton field 2) were not evident.

Pathogenicity scores from pair-wise matching of Fusarium strain and lettuce cultivar can be seen in Fig 1. Lettuce cultivar susceptibility rankings were previously known from field data and are listed in the Fig. 1 from low susceptibility of Meridian (blue) to high susceptibility of 1221 (red). Fusarium isolate virulence ranking were not previously known, but a cursory observation was made in preliminary trials, that being that 1306 and 1323 were weakly virulent (but still pathogens, blue) and that 1369 and 1370 were highly virulent (red).

The various combinations of pathogen and host cultivar presented in Fig 1. covered all potential interactions. Some combinations paired a pathogen of low virulence with a lettuce cultivar of low susceptibility (high tolerance), resulting in little disease (blue). Other combinations paired a pathogen with high virulence with a cultivar with high susceptibility (low tolerance), resulting in high levels of disease (red). Importantly, field-based rankings of susceptibility obtained in earlier field trials strongly correlated with the same rankings obtained in the greenhouse. Moreover, the greenhouse rankings were obtained testing lettuce cultivars against a range of Fusarium strains, thus making findings more comprehensive.

This type of study also provided a more detailed description of pathogen virulence. While large differences in pathogen virulence can be obtained qualitatively testing against one or two lettuce cultivars, finer differences can be obtained when testing against a selection of lettuce cultivars and comparing this profile against profiles of other FOL strains. Overall, greenhouse ratings of lettuce susceptibility and pathogen virulence can be obtained very efficiently under greenhouse conditions.

Discussion. There are several notable findings from this study. First, this study revealed that greenhouse-based pathogenicity testing can easily be executed and the findings correlate closely with those data obtained from field-based studies. This is important because greenhouse studies

can be conducted year-round and in close succession, rapidly advancing any research program's. objectives.

Second, it was readily apparent that there are a range of virulences among field isolates of FOL. It is known that there is only Race 1 in Arizona and California. However, this study revealed that even within Race 1, field isolates behave differently when challenged against a different host cultivar. This is also a very important finding because it suggests that to breed a tolerant cultivar that is robust, it must be tested against a range of FOL genotypes to ensure its tolerance is comprehensive. Again, this can be accomplished much more efficiently in the greenhouse as opposed to field-based trials.

And third, it was apparent that cultivar susceptibility was not just a simple response against a standard pathogen strain. Each cultivar responded differentially to different FOL strains, and that the cultivar response to a strain could not always be predicted from a neighbor cultivar response. Statistically speaking, there was a strong interaction between FOL strain and lettuce cultivar; each interaction being distinct and somewhat unique.

For this research, we also established protocol for preparing field soil for use in the greenhouse. While pathogenicity ratings were obtainable using commercial potting soil, our study found that using field soil mixed with vermiculite resulted in more disease development and a greater range of disease responses. Moreover, we developed protocol for inoculating un-infested field soil with FOL to mimic soil-based disease pressure. Further, we established protocol for inoculating lettuce seedings into non-infested soil for use in greenhouse-based FOL pathogenicity trials. Taken together, these findings and protocol development have prepared the way for a large expansion of greenhouse-based research on FOL management. In addition to cultivar screening, pathogen testing, and advancing our understanding of FOL biology and ecology, robust greenhouse-based trials can also be used to evaluate commercial and experimental products for efficacy in suppressing or preventing disease development and to evaluated certain cultural practices for their efficacy in disease suppression. The utility of greenhouse-based experiments in FOL research is far-reaching.

Table 1. Strains and cultivars selected for the current study. Field pathogenicity score for each cultivar is based upon trials conducted in 2007, 2013, 2014, and 2016.

Strain Number	year collected	location collected		Cultivar Tested	Field pathogenicity *ranking (most tolerant = 1)
BMP 1306	2017	Wellton		Meridian	**1**
BMP 1323	2017	Yuma		Oracle	**2**
BMP 1331	2018	Wellton		Icepalace	**3**
BMP 1339	2018	Yuma		Icecastle	**4**
BMP 1342	2019	Yuma		16527	**5**
BMP 1353	2019	Yuma		15537	**6**
BMP 1363	2019	Wellton		Raider	**7**
BMP 1367	2019	Yuma		1637	**8**
BMP 1369	2019	Wellton		7101	**9**
BMP 1370	2019	Wellton		1221	**10**

Fig 1. Cultivars tested against select Fusarium isolates. Score =; 1-no disease, 2-minor wilt on 1-2 leaves, 3-wilt on most leaves, 4-wilt on most leaves and necrosis, 5-dealth. Color change (blue to red) matches lettuce cultivar susceptibility, Fusarium strain virulence, and disease rankings for combinations.

Cultivar tested					Strain number					
	BMP 1306	BMP 1323	BMP 1331	BMP 1339	BMP 1342	BMP 1353	BMP 1363	BMP 1367	BMP 1369	BMP 1370
Meridian	1	1	1	1	1	1	2	2	2	2
Oracle	1	1	1	1	1	1	2	2	2	2
Icepalace	2	2	3	3	3	2	3	4	3	4
Icecastle	2	2	3	3	3	3	3	4	4	4
16527	2	2	3	3	3	3	3	3	3	3
15537	2	3	3	3	4	3	4	3	3	3
Raider	3	5	4	4	4	4	4	5	5	5
1637	3	4	4	4	5	5	5	5	5	5
7101	4	4	5	5	5	5	5	5	5	5
1221	3	4	5	5	5	5	5	5	5	5