Project Title: Race diversity and the biology of the spinach downy mildew pathogen.
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Summary
In conjunction with the International Working Group on Peronospora (IWGP) on spinach, the deviating isolate UA4410 was evaluated in a ringtest and nominated as race 14. Thus, there currently are 14 recognized races as a result of the Correll/Koike collaborative effort supported by the CLGRB. The Correll and Koike labs have continued efforts to survey and characterize isolates of the downy mildew pathogen in 2012. A total of 82 isolates were received and evaluated in 2012. Out of the isolates received, the race identifications of 54 isolates were documented. The majority of the isolates examined (>75%) were race 12, 13, or 14. Additional deviating isolates are also currently being evaluated on a number of newer spinach hybrids with putative race 1-14 resistance. Some of these lines are susceptible to some of the deviating isolates. In particular, one deviating isolate (UA4712) from multiple locations in 2012 was able to infect some R1-14 resistant lines. This isolate needs to be further evaluated to determine if it should be nominated as a new race. Progress has been made in evaluating a soil-less downy mildew screen to help expedite the simultaneous characterization of multiple isolates. In addition, several organic and conventional seed treatments are being evaluated to determine the efficacy of these materials for delaying the onset of downy mildew symptoms on seedlings.

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Abstract

In conjunction with the International Working Group on Peronospora (IWGP) on spinach, the deviating isolate UA4410 was evaluated in a ringtest and nominated as race 14. Thus, there currently are 14 recognized races as a result of the Correll/Koike collaborative effort supported by the CLGRB. The Correll and Koike labs have continued efforts to survey and characterize isolates of the downy mildew pathogen in 2012. A total of 82 isolates were received and evaluated in 2012. Out of the isolates received, the race identifications of 54 isolates were documented. The majority of the isolates examined (>75%) were race 12, 13, or 14. Additional deviating isolates are also currently being evaluated on a number of newer spinach hybrids with putative race 1-14 resistance. Some of these lines are susceptible to some of the deviating isolates. In particular, one deviating isolate (UA4712) from multiple locations in 2012 was able to infect some R1-14 resistant lines. This isolate needs to be further evaluated to determine if it should be nominated as a new race. Progress has been made in evaluating a soil-less downy mildew screen to help expedite the simultaneous characterization of multiple isolates. In addition, several organic and conventional seed treatments are being evaluated to determine the efficacy of these materials for delaying the onset of downy mildew symptoms on seedlings.

Objectives:

1. Maintain the UCCE downy mildew race identification service in California and screen contemporary germplasm to predominant races in California. Identify and characterize new races that might occur.

2. Develop and examine a soil-less downy mildew test for spinach similar to the magenta-box screen used for lettuce downy mildew.

3. Examine organic products (both commercial and experimental) for their effectiveness in reducing downy mildew on spinach.

4. Evaluate seed treatments and drench applications for effectiveness in controlling downy mildew and for the longevity of control. Standardize a test to establish baseline levels of sensitivity to metalaxyl of the downy mildew pathogen.

Procedures:

1. Maintain the UCCE downy mildew race identification service in California and screen contemporary germplasm to predominant races in California. Identify and characterize new races that might occur.

Our established protocol was used to inoculate a standardized set of spinach differentials to evaluate disease reactions and determine race identification. Isolates typically are evaluated during a 2-3 week time frame and any isolates not conforming to previously identified races are evaluated in additional inoculation tests. In some cases, multiple inoculations are performed to separate field samples where there appear to be mixtures of different races in the same sample. Intermediate reactions on a given differential (infection levels of > 15% but < 85%) often indicate that there may be a mixture of races in the field sample. If a mixture is suspected in the field sample, inoculum from the first round of evaluations is collected and used to inoculate two separate spinach hybrids that have a different resistance spectrum. Subsequently, inoculum from these two different hybrids is harvested and used in separate inoculation tests on the set of spinach differentials. During this project period, samples were received from throughout the coastal production area of California as well as from Arizona (Yuma).

2. Develop and examine a soil-less downy mildew test for spinach similar to the magenta-box screen used for lettuce downy mildew.

Thus far, a number of soil-less methods have been evaluated for use in screening spinach downy mildew, similar to the one used for downy mildew on lettuce. The detached leaf assays were performed as follows:

Inoculum of the spinach downy mildew pathogen was prepared by placing 4-5 infected leaves in water, agitating the mixture, and filtering the suspension through cheesecloth. This suspension was used for the inoculation assays. For method one below, the sporangia suspension was placed on the surface of a water agar Petri dish. For methods two and three, 4-5 drops of the suspension were placed on the leaf surface. For each plate, 3-4 leaves were inoculated on the top surface and 3-4 leaves on the bottom surface.

A. The first method followed a protocol developed for downy mildew of Quinoa (a close relative of spinach) whereby leaves were laid down on the surface of a water agar Petri dish that had been sprayed with inoculum.

B. A second method was described by Frinking et al, 1985. In this method, cotyledons and true leaves were placed in the Petri dishes, inoculated, and then covered with two layers of wet filter papers. The dishes were incubated in the dark at 18 C overnight and then placed in the growth chamber set at 16 hours light/8 hours dark at 20 C.

C. A third method was similar to method 2 but kinetin (a cytokinin plant hormone) was added to the filter paper at 1ug/ml final concentration to help preserve the detached leaves.

3. Examine organic products (commercial and experimental) for their effectiveness in reducing downy mildew on spinach.

4. Evaluate seed treatments and drench applications for the effectiveness in controlling downy mildew and for the longevity of control. Standardize a test to establish baseline levels of sensitivity to metalaxyl of the downy mildew pathogen.

For objectives 3 and 4, a wide range of materials, including organic treatments, foliar sprays, and seed treatments, continue to be evaluated. For the organic treatment experiments, spinach was planted into trays and grown until approximately the 3-4 leaf stage. A number of experimental and commercial products were prepared as foliar sprays according to product instructions. The materials were applied using an airbrush sprayer the spinach in approximately 250 ml of water. One application was made and then the spinach was inoculated with a sporangial suspension once the spray had dried. Plants were then incubated in a dew chamber for 24 hrs (18 C and 100% RH) and then moved to a growth chamber (18-20 C with 16/8 hr light/dark cycle). A control set of spinach was sprayed only with distilled water prior to inoculation. After six days, plants were returned to the dew chamber for 24 hrs to induce sporulation and then evaluated for downy mildew disease 7 days after the initial inoculation.

For seed treatment experiments, two spinach lines (2207 and 2208) were treated with metalaxyl at US (0.417 ml / kg seed) and EU (2.0 ml / kg seed) rates, Actinovate (6 oz. / 100 lbs of seed), or water (1.5 ml / 50 g seed) as a control treatment. When the spinach was at the 2-4 true leaf stage, plants were inoculated by spraying them with a sporangial suspension of a race 12 isolate (UA2209). Plants were incubated and evaluated for disease as previously described. In a second experiment, US and EU metalaxyl seed treatments are being compared with seed treated by Germains seed treatment, metalaxl + Thiram combination, and a water control.

Results and Discussion:

Objective 1

A total of 82 field samples, primarily from California and Arizona, were received and evaluated in 2012. The majority of the isolates (>75%) were identified as either race 12, 13, or 14, with race 13 being the most frequently recovered race (Fig. 1). A number of deviating isolates with a novel virulence phenotype were identified in 2012. One deviating isolate in particular, UA4712, was recovered from multiple locations in 2012 and was able to infect some R1-14 resistant lines. This isolate needs to be further evaluated to determine if it should be nominated as a new race. Frequencies of races 10, 11, 12, 13, and 14 over the past five years throughout California and Yuma Arizona are shown in Figure 2.

Figure 1. Frequency of races identified in 2012.

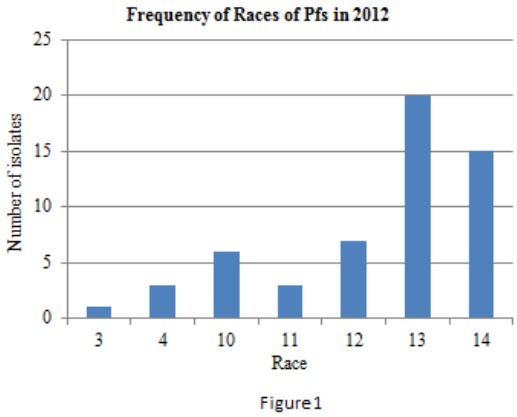
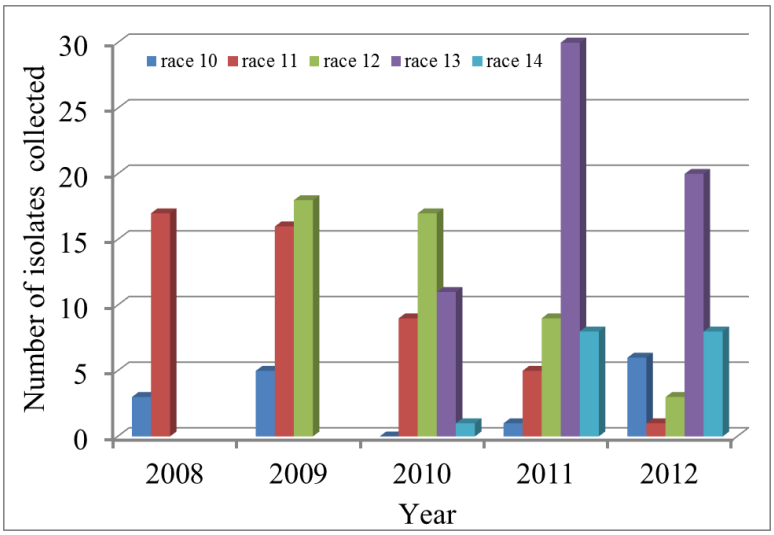


Figure 2. Frequencies of races identified from 2008 to 2012.



Unlike in years past, we have detected mixtures of two or more races from some individual fields. This occurrence complicates the identification of races and the interpretation of the resistance of individual lines. Another interesting observation is that in spring 2013, a number of spinach samples were received at the UCCE lab in which disease symptoms (yellow lesions) were extensive but corresponding signs (purple sporulation) were sparse or absent. In such cases, the tests for the downy mildew races were always negative due to lack of spores.

The deviating isolates that have been observed have been able to overcome certain resistance loci and various combinations of resistance in commercial hybrids. We are continuing to evaluate these deviating isolates to determine which of the hypothesized resistance loci are most effective

for these deviating isolates (Tables 1 and 2). We have also screened a large set of germplasm against races 10-14 and several of the deviating isolates.

Table 1. Isolates recovered and race determinations.

Isolate	Host cultivar	Origin	Date received	Race ID	Date tested
UA0312A	Tasman	CA	1/18/2012	13	1/25/2012
UA0312B	Tonga	CA	1/19/2012	12	1/31/2012
UA0612A	Spiros	CA	2/7/2012		
UA0612B	Racoon	CA	2/7/2012		
UA0612C	Racoon	AZ	2/8/2012		
UA1012A	Yabi	AZ	3/7/2012	not viable	
UA1012B	Toucan	CA	3/9/2012	novel	
UA1312		NL	3/28/2012	novel	4/4/2012
UA1412	Pigeon	AZ	4/5/2012	14	5/10/2012
UA1512A	Cello	AZ	4/10/2012	13	5/9/2012
UA1512B	Tambourine	AZ	4/10/2012	12 or 14	4/18/2012
UA1512C	Molakai	CA	4/11/2012	not viable	4/25/2012
UA1512D	Molakai	CA	4/13/2012	not viable	5/2/2012
UA1612A	PV1388	CA	4/17/2012	4	5/8/2012
UA1612B	Chevelle	AZ	4/17/2012	4	4/24/2012
UA1812A	Cello	CA	5/3/2012	14	5/10/2012
UA1812B	Amazon	CA	5/3/2012	14	5/17/2012
UA2012	Solomen	CA	5/15/2012	10	5/22/2012
UA2112	Unipack 12	NJ	5/24/2012	3	5/30/2012
UA2312		CA	6/7/6012	13	
UA2412				13	6/22/2012
UA2612	Pigeon	CA	6/29/2012	novel?	7/13/2012
UA4612	Molakai/aikers	CA	11/14/2012	mixture	
UA4712	Chevelle	CA	11/20/2012	4?	
UA5212	Corvair	AZ	12/27/2012	12	1/24/2013

Table 2. Disease reactions of races 10-14 and deviating isolates to individual lines and/or resistance loci.

Line	**Gene**	**10**	**11**	**12**	**13**	**14**	**UA4711 (Spain)**	**UA1312 (Netherlands)**	**UA1012B (Toucan)**	**UA2612 (Pigeon)**	**UA4712 (Chevelle)**
Resistoflay	**R5**	+	+	+	+	+	+	+	+	+	+
Califlay	**R3**	+	-	-	+	-	+	-	-	-	+
Bolero	**R4**	+	+	+	+	+	-	+	+	+	-
Campania	**R6**	+	-	+	**?**	+	-	-	+	-	-
NIL1	**R1**	+	-	+	-	+	+	-	+	-	-
NIL2	**R2**	-	+	+	+	+	-	+	-	+	-
Pigeon		-	-	-	-	+	-	+	-	+	-

One deviating isolate that has been observed from a number of locations, UA4712, was evaluated on some newer lines with putative resistance to races 1-14 lines. Spinach cultivars Coati and Meerkat and line 376/10 appear to be resistant to this deviating isolate (Table 3).

Table 3. Disease reactions of some lines to the deviating isolate UA4712.

Cultivar	Disease Reaction
PV-1388	+
PV-2395	+
376/10	-
Caledonia	+
Coati	-
Meerkat	-
E03D.0579	+

Objective 2

A. For the Quinoa method (leaves were laid down on the surface of a water agar Petri dish that had been sprayed with inoculum) leaves were observed for infection and sporulation over a 2 week period. Although some yellowing and chlorosis of leaves was observed, no evidence of infection was present and no sporulation was observed on the plants during the observation period.

B. For the Frinking method (cotyledons and true leaves were placed in the Petri dishes, inoculated, and then covered with two layers of wet filter papers; (Fig. 3) the sporangia did germinate on the leaf surface but they did not continue to develop or infect the leaf.

C. For detached leaves in which kinetin was added to the filter paper, again, the leaves stayed green and turgid longer, but no infections were observed. We are continuing to evaluate this procedure by manipulating the temperature and incubation conditions.

Although it is not a soil-less method, we have been able to work with multiple isolates within a growth chamber to expedite the number of isolates that can be characterized by using isolation domes to cover inoculated plants (Fig. 4). The domes effectively isolate inoculated plants and prevent cross-infection when multiple isolates of the pathogen are being evaluated. In addition, the UCCE lab is discussing the possible expansion of the spinach downy mildew program via industry support for a growth room.

Figure 3. Demonstration of a soil-less incubation of spinach leaves inoculated with downy mildew spore suspension.

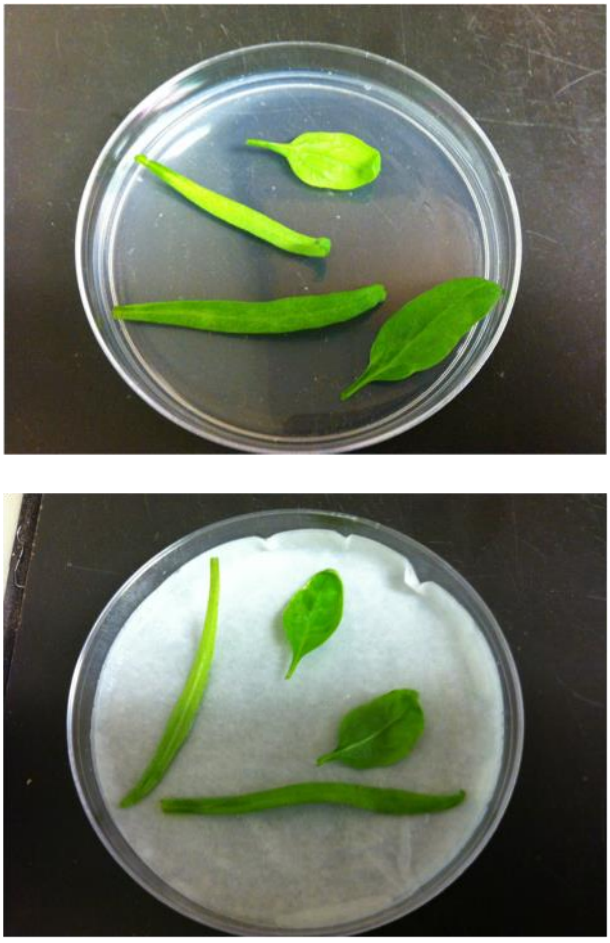


Figure 4. Dome isolation trays to allow multiple isolates of the pathogen to be evaluated simultaneously and prevent cross-contamination of isolates.

Objectives 3 and 4

Thus far, the organic materials evaluated have not shown any efficacy in the greenhouse downy mildew assay (Table 4). These treatment plus inoculation experiments appear to mirror the field results of growers and pest control advisors, in which they report poor performance of organically approved products used to manage spinach downy mildew.

Table 4. Efficacy of organic foliar spray on spinach downy mildew disease

Treatment	% infection
Sonata	100
Nordox	100
Actinovate	100
Green and Grow	100
Serenade	100
T-22	100
ddH2O	100

For seed treatments, all treatments were susceptible and no effect of metalaxyl seed treatment was observed on downy mildew (Table 5). This result was unexpected due to the apparent delay in downy mildew observed under field conditions. As a result, the tests are being repeated and commercially treated seed is also being evaluated. Currently, the metalaxyl/Germains/metalaxyl + Thiram experiment is in progress (Table 6). The efforts will result in developing baseline information to evaluate the sensitively of isolates of the downy mildew pathogen to metalaxyl.

Table 5. Impact of seed treatments on spinach downy mildew disease

Cultivar	Treatment	% infection
2207	ddH2O	100
	Actinovate	100
	US Metalaxyal	100
	EU Metalaxyal	100
2208	ddH2O	100
	Actinovate	100
	US Metalaxyal	100
	EU Metalaxyal	100

Table 6. Ongoing experiment on impact of seed treatments on spinach downy mildew disease

No.	Line	Seed Treatment	Source
1	2207	Water treated	Germains
2	2207	EU Metalaxyl	Germains
3	2207	US Metalaxyl	Germains
4	2208	EU Metalaxyl	Germains
5	2208	US Metalaxyl	Germains
6	Lazio	Untreated	
7	Ashley	Thiram/Metalaxyl	Holaday
8	Ebro	Thiram/Metalaxyl	Holaday
9	Lazio	Thiram/Metalaxyl	Holaday
10	Platypus	Untreated	Holaday

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