Project Title: Race diversity and the biology of the spinach downy mildew pathogen
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Cooperating Personnel: Growers, pest control advisors, and seed company personnel in the spinach growing regions in California and Arizona.

Abstract: Spinach downy mildew was especially challenging for California and Arizona growers in 2013 and 2014. In most other years, typically only one or two races of the downy mildew pathogen (*Peronospora farinosa* f. sp. *spinaciae* [Pfs]) predominated in a given area. However, our most recent work indicated that multiple races, often as many as 4-5 different ones, have been recovered from a single growing area. The occurrence of multiple races in a given area likely is due to the use of a wide range of spinach hybrids with different genetic resistance backgrounds to the pathogen. An unusually large number of samples examined in 2013-2014 also were mixtures of at least two different races. We continue to focus on evaluating isolates of the pathogen collected throughout California for their race identity and to evaluate novel strains for how they deviate from previously described races. Currently, 14 races of the Pfs pathogen have been described and the International Working Group on Peronospora (IWGP) is currently evaluating a deviating strain to be nominated as race 15 (UA4712). In addition to UA4712, deviating isolates that have been examined in detail and are noteworthy include UA1414 (also designated ES1314 from Spain), UA1012, UA1312, UA2213, UA1014, and UA1914. Progress has been made in evaluating a soil-less downy mildew screening method to expedite the simultaneous characterization of multiple isolates. However, this system is not yet a viable alternative to the screening process due to logistical difficulties in setting up and maintaining plants and in evaluating plants for susceptibility. Finally, we continue to evaluate organic and conventional seed treatments and to develop a robust baseline test for evaluating the efficacy of metalaxyl seed treatments.

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 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 effectiveness in controlling downy mildew and for 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 to the spinach with an airbrush sprayer using approximately 25 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 the Germains seed treatment, metalaxl + Thiram combination, and a water control.

Additional treatments on two hybrids, 65 and 7132, were also evaluated. These included a non-treated control, an EU standard seed treatment, and metalaxyl seed treatments at both EU and US rates.

Results and Discussion:

Objective 1

The 2013-2014 spinach season was particularly challenging due to the occurrence of multiple races in a given area at a given time. Because of the unpredictability of which race, or races, may be present, many growers are following a sound management practice of growing a wide range of spinach varieties that have diverse genetic backgrounds with regard to mildew resistance. As a result, numerous races have been active. For example, it was not uncommon to identify races 10 through 14 all in a given area at a given time (Fig. 1). In addition, four deviating strains, namely UA4712, UA1312, UA1514B, and UA1012, were identified from numerous samples (Fig 1.). UA4712 was initially identified in 2012 and has been found in a number of locations in 2013 and 2014 (Table 1). This strain is similar to race 4 according to the disease reactions of the differential cultivars, but deviates in that it can attack some race 1-14 resistant lines. A ringtest is currently underway with the IWGP to nominate this as a new race. In addition UA1312, another deviating isolate first identified in 2012, has been found at numerous locations and on a wide range of contemporary cultivars (Tables 1, 2, and 3). This isolate is similar to race 11 with regard to disease reactions on the differential spinach lines, but it can attack the variety Pigeon and other lines with similar genetic backgrounds. Isolate UA1514 was similar to UA1312 but was able to infect Campania (Table 3). A third deviating isolate, UA1012, also identified in 2012, continues to be found but many contemporary spinach varieties have resistance to this strain.

In 2013, an isolate designated UA2213A was identified and was of particular concern in that it appeared to infect all known resistances with wide range of genetic backgrounds (Table 4). After further detailed examination by growing the isolate on a variety with specific resistance (Meerkat), it was determined that this was a mixture of races and not a deviating strain as originally identified. However, we have focused on a few additional isolates, namely UA1014 and UA1914, that initially have been able to infect a wide range of resistances (Table 4). Efforts continue to thoroughly dissect these strains by increasing them on varieties with different resistances (Table 4) to determine if they are deviating strains or complex mixtures.

Objective 2

Work continues to evaluate soil-less plant production and inoculation procedures. However, results from both the previous year and this year indicate that, although approaches have worked, they do not necessary save time or resources. In addition, the disease evaluation procedures are somewhat problematic in that a clear susceptible or resistant phenotype can be difficult to determine and thus reduces the confidence in the resistance evaluation.

Objectives 3 and 4

Thus far, the organic materials evaluated have not shown any efficacy in the greenhouse downy mildew assay (Table 5). 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 against spinach downy mildew.

We are continuing to evaluate the efficacy of metalaxyl seed treatments for downy mildew management. The metalaxyl seed treatments, both at the US and the higher EU concentration rates, are being evaluated under greenhouse and field conditions. Due to concerns about the consistency of deposition of the materials onto spinach seed, these experiments are being repeated.

Figure 1. Frequency of races examined in 2013-2014.

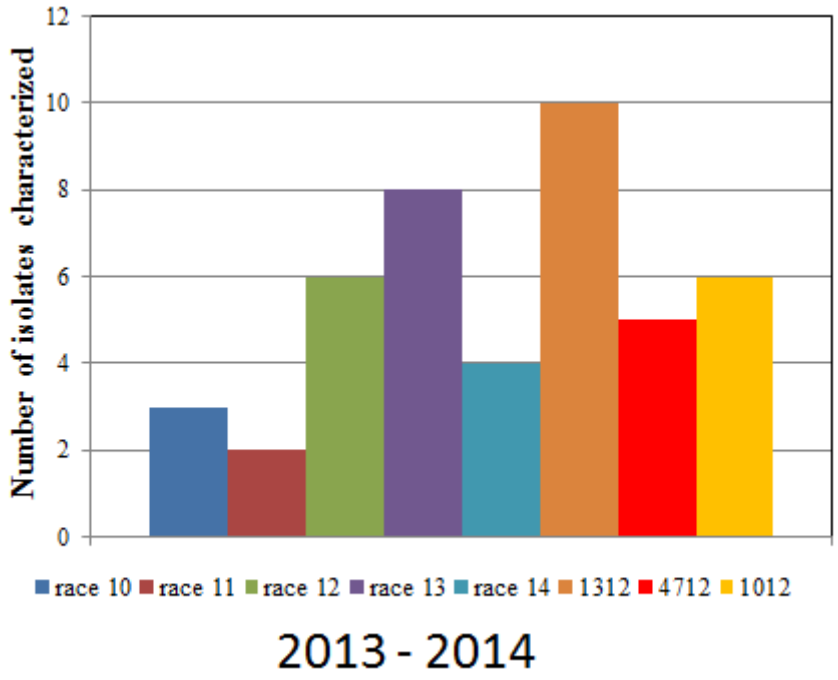

Table 1. Disease reactions of several deviating isolates examined in detail in 2013-2014.

Cultivar	UA4712	UA1414 (ES1314)	UA1414B (Old US 4)	UA1012	UA1312
Viroflay	+	+	+	+	+
Resistoflay	+	+	+	+	+
Califlay	+	+	+	-	-
Clermont	-	+	-		
Campania	-	-	-	-	-
Boeing	-	-	-	+	-
Lion	-	-	-	-	-
Lazio	-	-	-	-	+
Whale	+	+	+		
Pigeon	-	-	-	-	+
Caladonia	+	+	-		-
Coati	-	-	-		-
E03D.0579	+	+	-		-
Mandril		-	-		
Meerkat	-	-	-		-
Platypus	-	-	-		
Plover		-	-		
PV1053		+	-		-
Scorpius		-	-		
Woodpecker		-	-		
SSR-SP-29		-	-		
PV1043	+				-
PV1047	+				-
PV1388	+				-
PV2395	+				-
NIL1				+	-
NIL2				-	+

[1] UA4712 caused similar disease reactions on differentials as race 4; however, Caladonia is susceptible and likely can overcome *RPF*7.
UA1414A (= ES1314, an isolate from Spain) is similar to UA4712 except it caused disease on Clermont (*RPF*4).
UA1414B is an older isolate of US Pfs race 4.
UA1012 can overcome *RPF*1 but not *RPF*2 and *RPF*3.
UA1312 is like race 11 except Pigeon is susceptible.

Table 2. Isolates identified on contemporary cultivars that have the UA1312 type disease reactions.

Isolate	Host Cultivar	Origin	Date Received	Result	Location
UA1312	unknown	NL	3/28/12	1312	Netherlands
UA1613B	Minorca	FL	4/18/13	1312	Indiantown, FL
UA3713	Dromedary	CA	9/12/13	1312	Soledad, CA
UA4113A	Plover	CA	10/10/13	1312	King City, CA
UA0514	Platypus	AZ	1/29/14	1312	Yuma, AZ
	Lancerote	CA	4/21/14	1312	Monterey Co., CA
	Meerkat	CA	4/11/14	1312	Monterey Co., CA
	Gazelle	CA	4/18/14	1312	unknown
	Trumpet	CA	4/24/14	1312	San Benito Co., CA
UA1514B*	Gazelle	CA	4/8/2014	1514B	Monterey Co., CA

*UA1514B is similar to UA1312 except Campania is susceptible to this isolate but resistant to UA1312.

Table 3 Disease reactions of differentials to UA1312 and UA1514B.

Differential	UA1312	UA1514B
Viroflay	+	+
Resistoflay	+	+
Califlay	-	-
Bolero	+	nt
Clermont	nt	+
Campania	-	+
Avenger	-	-
Lion	-	-
Lazio	+	+
Dolphin	-	-
Whale	nt	-
Pigeon	+	+
Polka	-	nt
NIL1	-	-
NIL2	+	+

UA1514B is similar to UA1312 except Campania is susceptible to this isolate but resistant to UA1312. nt = not tested.

Table 4. Additional deviating strains of *Pfs* of particular concern.

	UA2213A	UA2213APM	UA2213AP3	UA1014A	UA1014APL	UA1014APLP	UA1914E
Viroflay	+	+	+	+	+	+	+
Resistoflay	+	+	+	+	+	+	+
Califlay	+	+	+	+	+	+	+
Clermont				+	+	+	+
Campania	+		+	+	+	+	+
Boeing				+	+	+	+
Lion	+	-	-	+	+	+	+
Lazio	+	+	+	+	+	+	+
Whale			+	+	+	+	+
Pigeon	+	-	+	+	+	+	+
Caladonia	+			+	+	+	+
Coati	+	-		+	+	+	+
E03D.0579	+	-		+	+	+	+
Mandril	+			+	+	+	+
Meerkat	+	-		+	+	+	+
Platypus	+			+	+	+	+
Plover	+			+	+	+	+
PV1053	+			+	+	+	+
Scorpius	+			+	+	+	+
Woodpecker	+			+	+	+	+
SSR-SP-29	+			+	+	+	+
Bolero	+		+				
Avenger	+	-	-				
Dolphin	+	-	-				
NIL1	+	-	-				
NIL2	+	+	+				
PV1043	+	-					
PV1047	+	-					
PV1388	+	-					
PV2395	+	-					
376/10	+	+					

The field isolate UA2213A initially infected all differentials. UA2213APM (purified on Meerkat) was similar to race 13 indicating UA2213 was a mixture of races. UA2213AP3 (purified on Califlay) was similar to race 13 except Pigeon was susceptible to this isolate. Both UA2213APM and UA2213AP3 were only tested once.

Isolates UA1014A and UA1914E infected all lines tested. UA1014APL, derived from UA1014A purified on Lion, infected all differentials. The reactions on Meerkat, Scorpius, and Woodpecker need to be further tested to confirm reactions. UA1014APL was further purified on Pigeon (UA1014APLP) and inoculated onto the differentials, and is currently being evaluated along with a diverse PI collection of spinach germplasm.

Table 5. Efficacy of organic foliar sprays on spinach downy mildew

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

* ddH2O = double distilled water control

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For more detail on the research supported in part by the CLGRB, please see the following scientific publications:

Feng, C., Correll, J. C., Kammeijer, K. E., and Koike, S. T. 2014. Identification of new races and deviating strains of the spinach downy mildew pathogen *Peronospora farinosa* f. sp. *spinaciae*. Plant Disease 98:145-152.

Feng, C., Mansouri, S., Bluhm, B. H., du Toit, L. J., and Correll, J. C. 2014. Multiplex real-time PCR assays for detection of four seedborne spinach pathogens. Journal of Applied Microbiology. DOI: 10.1111/jam.12541.

Please e-mail Jim Correll or Steve Koike if you would like a PDF copy of the above articles.