

**Project Title: Race diversity and the biology of the spinach downy mildew pathogen.
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Abstract:

Spinach downy mildew continues to be a major production constraint for growers in California and Arizona in 2014 and early 2015. Disease incidence and severity were high, particularly in organic fields. Although we continue to identify known races such as 10, 11, 12, 13, 14, and 15 from numerous fields this past year in both conventional and organic fields, several deviating isolates also have been identified, including a UA1014APLP type. This isolate type can infect the cotyledons and true leaves of the standard differentials, and the cotyledons, but not the true leaves, of a number of hybrids with reported race 1-15 resistance. A total of 6 isolates with this reaction type have been identified in California and Arizona this past year (UA1014APLP from Coati, UA1914E from Silver Whale, UA3414G from Coati, UA2015-13 from Rigel, and UA2015-16 from Carrera). Work continues on this deviating type as it has potential to cause considerable damage to some of the new hybrids being released. Another deviating isolate type, UA2015-11A, also was identified. This isolate type could only infect Viroflay, Resistoflay, and Pigeon of the differentials, but was able to infect several of the newer race 1-15 resistant hybrids such as Coati, Plover, Platypus, Meerkat, and Woodpecker. Also, a wide range of contemporary spinach varieties were evaluated in an extensive field trial in Yuma, AZ in early 2015 under natural disease pressure. At this same location, a large-scale trial was conducted to evaluate the efficacy of biofungicide materials relative to conventional fungicides under heavy disease pressure.

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 continue to develop. The UCCE group generally processes field samples from throughout the state for race identification and serves as a frontline service to the industry. The University of Arkansas complements this effort by also doing some field isolate identification, but also focuses

on deviating isolates and isolates that potentially represent novel races. Novel, or deviating, isolates typically take 3-6 months of repeated inoculations to verify how they may deviate from described races. A large number of field isolates were recovered in 2014-2015 seasons that were identified as one of the previously described races. However, we identified a number of novel isolates, including one that we determined to be able to infect the cotyledons of all of the international differentials, but not the true leaves of several differentials. We need to determine how common this novel type is, if the differentials reactions are temperature sensitive, and if indeed this represents a unique isolate that potentially should be nominated as a new race.

2. We continually screen a large set of spinach germplasm from plant introduction collections (Pi's) as well as advanced breeding lines and commercially released material for resistance to various contemporary races of the downy mildew pathogen. This effort helps determine which know resistances are effective to the various races.

3. We are evaluating experimental and commercial biofungicide materials for efficacy in use in organic production settings. The biofungicides are evaluated in a greenhouse test and are being tested under different temperature conditions to determine if efficacy and temperature are related.

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.

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. From April 2014 through March 2015, downy mildew samples from a wide range of production areas in California and Arizona (Yuma) were recovered and processed by our two laboratories.

2. Screening commercial spinach hybrids and advanced lines for disease reactions to contemporary and deviating isolates of the downy mildew pathogen.

We continually screen a large set of spinach germplasm from plant introduction collections (Pi's) as well as advanced breeding lines and commercially released material for resistance to various contemporary races of the downy mildew pathogen. This effort helps determine which known resistances are effective to the various races. In addition, a large-scale variety trial was conducted in Yuma, Arizona in February of 2015 under heavy naturally occurring disease pressure.

3. Evaluation of commercial and experimental biofungicide materials for efficacy in managing downy mildew.

We are evaluating experimental and commercial biofungicide materials for efficacy in use in organic production settings. The biofungicides are evaluated in a greenhouse test and are being tested under different temperature conditions to determine if efficacy and temperature are related. In addition, a large-scale field trial was conducted in Yuma, Arizona in February 2015 comparing biofungicide materials with conventional materials for efficacy.

Results and Discussion

Objectives 1 and 2.

A total of 59 isolates were recovered and evaluated this past year. The major of isolates examined were identified as either race 10, 11, 12, 13, 14, or 15. Races 13, 14, and 15 were the most common races identified. The disease reactions of the 15 reported races are shown in Table 1.

Table 1. Disease reactions of the 15 known races of the spinach downy mildew pathogen on the standard set of differential spinach lines.

Differential cultivar	Race of <i>Peronospora farinosa</i> f. sp. <i>spinaciae</i>														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Viroflay	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Resistoflay	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Califlay	-	+	-	+	-	+	+	-	-	+	-	-	+	-	+
Clermont	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-
Campania	-	-	-	-	-	+	-	+	+	+	-	+	+/-	+	-
Boeing	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-
Lion	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Lazio	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-
Whale	-	-	-	(-)	-	(-)	(-)	-	-	+	-	+	+	-	+
Pigeon	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Caladonia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

+ = susceptible reaction with symptoms and evidence of sporulation.

- = resistant reaction with no symptoms or evidence of sporulation.

+/- = intermediate type reaction.

(-) = variable reaction depending on the test.

A number of deviating isolates also were identified this past year with the reaction type of UA1014 APLP being the one in which a considerable focus was devoted. This isolate “type” was identified among the following isolates UA1014APLP from Coati, UA1914E from Silver Whale, UA3414G from Coati, UA2015-13 from Rigel, and UA2015-16 from Carrera. The disease reactions of this type on the standard set of differentials as well as some additional lines reported to be resistant to races 1-15 are listed in Table 2. Two additional deviating isolates, UA2015-11A collected on Caelum, and UA2015-13C collected from Woodpecker, were also characterized. These deviating isolates were able to infect a number of the newer race 1-15 resistant varieties (Table 3).

A field trial was conducted in Yuma, AZ at the University of Arizona Experiment Station. A total of 73 commercial varieties were planted in replicated plots and evaluated for downy mildew disease incidence and severity in March. Excellent disease pressure was observed in the trial and a wide range in disease resistance among the varieties was observed (Figure 1 and 2).

Table 2. Disease reactions of the UA1014APLP type reaction on the cotyledons and true leaves of differentials and several race 1-15 resistant spinach lines.

Cultivar	UA1014APLP	
	Cotyledons	True Leaves
Viroflay	+	+
Resistoflay	+	+
Califlay	+	+
Clermont	+	+
Campania	+	+
Boeing	+	+
Lion	+	+
Lazio	+	+
Whale	+	+
Pigeon	+	+
Caladonia	+	-
Coati	+	-
E03D.0579	+	-
Meerkat	+	-
Platypus	+	-
Plover	+	-
PV1053	+	-
Scorpius	+	-
Woodpecker	+	-
SSR-SP-29	+	+

Table 3. Disease reactions of spinach differentials to isolate UA201511A.

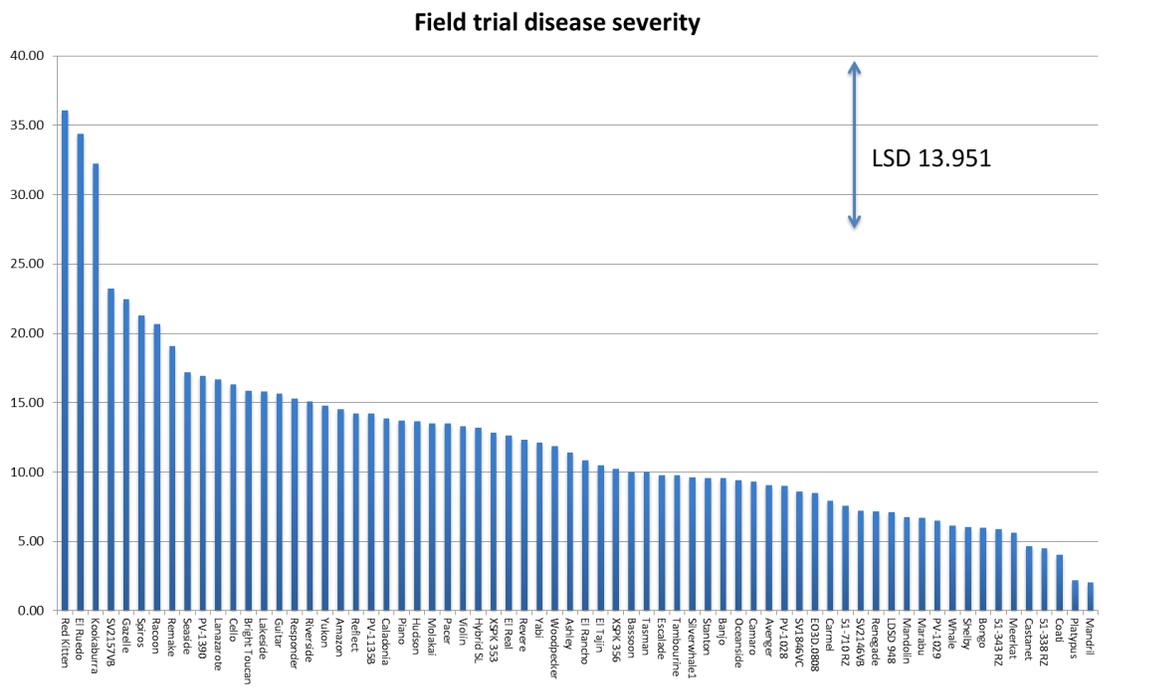
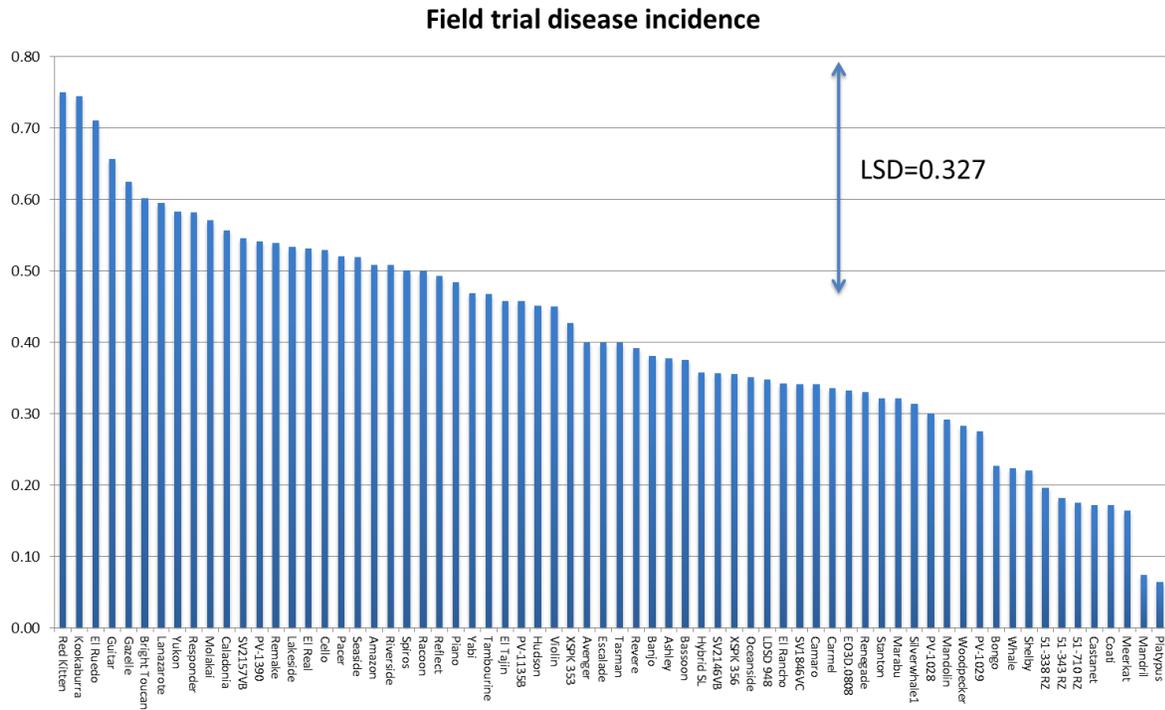
Cultivar	Disease Reactions	
	Cotyledons	True leaves
Viroflay	+	+
Resistoflay	+	+
Califlay	-	-
Clermont	-	-
Campania	-	-
Boeing	-	-
Lion	-	-
Lazio	-	-
Whale	-	-
Pigeon	+	+
Caladonia	-	-
Coati	+	+
E03D.0579	-	-
Meerkat	+	+
Platypus	+	+
Plover	+	+
PV1053	-	-
Scorpius	-	-
Woodpecker	+	+
SSR-SP-29	-	-

UA2015-11A was collected on Caelum from Yuma, AZ. Another isolate, UA2015-13C, with this same reaction type was recovered from Woodpecker.

+ = susceptible

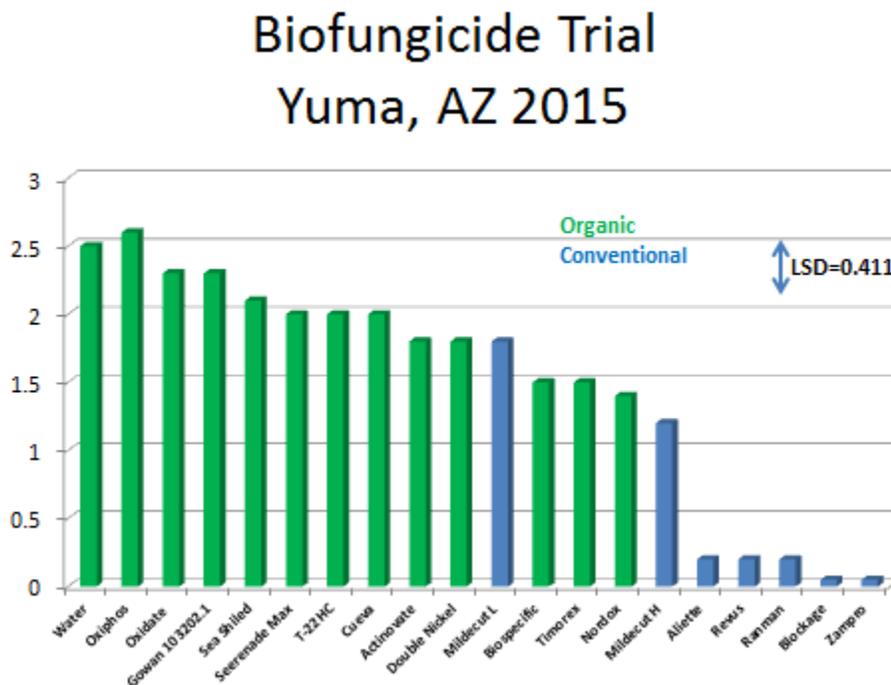
- = resistant

Figure 1. Disease reactions (disease incidence and disease severity) of commercial spinach hybrids in a field trial in Yuma, AZ in February 2015.



Objective 3. A large scale field trial was conducted to evaluate the efficacy of a number of commercial and experimental biofungicides for their ability to manage downy mildew on spinach. With an increase in organic spinach production, there is a great demand for a material to manage downy mildew in organic production. A total of 12 biofungicide materials were evaluated as were six conventional fungicides. Although some of the biofungicides resulted in a reduction in disease severity, none of the materials performed as well as the conventional fungicides (Figure 2, Table 3). Furthermore, the level of control provided by the biofungicides in this trial would not likely be commercially acceptable.

Figure 2. Evaluation of commercial and experimental biofungicide materials for efficacy in managing downy mildew.



Disease severity was rating on a scale of 0-4 with 0 = no disease; 1 = 1-25% disease leaves; 2 = 26-50%; 3 = 51-75%; and 4 = 76-100%

Table 3. Statistical evaluation of biofungicide and standard treatments.

Code	Treatment	Disease severity	Statistical comparison
T13	Oxiphos	54.2	a
T1	Water control	50.0	ab
T12	Oxidate 2.0 + AquaSil	45.8	ab
T6	Gowan 10320	41.7	abc
T7	Sea Shield	41.7	abc
T14	Cueva	37.5	abc
T8	Serenade Max	37.5	abc
T9	T-22HC	37.5	abc
T10	Actinovate	33.3	bcd
T15	Double Nickel	33.3	bcd
T20	Mildicut Low	33.3	bcd
T11	Biospecific	25.0	cd
T16	Timorex EC 33	25.0	cd
T5	Nordox ³	17.0	de
T19	Mildicut High	16.7	de
T3	Revus	2.0	e
T18	Ranman + Silwet L-77	1.7	e
T2	Aliette	1.7	e
T17	Zampro	0.7	e
T4	Blockade	0.3	e

Treatments followed by the same letter are not significantly different ($p = 0.05$)

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For more detail on the spinach research and 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.

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