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 through the first part of 2016 with very high disease incidence and severity in the desert production areas, particularly under organic production. Several conventional fields were also observed with high levels of downy mildew, but in most cases, these fields had some fungicide application issues whereby fields were either not sprayed or not sprayed with effective materials.

Over 150 inoculations were conducted up through March 2016 to examine the race diversity of the downy mildew in California and Arizona. The majority of the isolates tested were identified as UA2015-19B type. This isolate was recently nominated as race 16 based on a cooperative ringtest with participants of the International Working Group on Peronospora (IWGP) that was initiated by our laboratories. A press release announced the nomination of race 16 in March, 2016 (http://ucanr.edu/blogs/SalinasValleyAgriculture/index.cfm).

Although we continue to identify known races such as 4, 10, 11, 12, 13, 14, 15, and 16 from numerous fields this past year in both conventional and organic fields, several deviating isolates also were identified. As of early 2016, 10 isolates of the deviating strain UA1014APLP have been identified. 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. The UA1014 type isolate has now been identified in 2014, 2015, and 2016

from CA, AZ, and TX on 10 different cultivars (Coati, Silverwhale, Platypus, Rigel, Carrera, Siena, SP966, and Goldeneye). Work continues on this deviating type as it has potential to cause considerable damage to some of the new hybrids being released. This isolate type was evaluated in a ringtest by the IWGP in the summer of 2015 but was not nominated as a novel race type at that time. This isolate type should be reevaluated for consideration as a novel race. Several isolates recovered from race 1-16 resistant material are currently being evaluated in race tests.

Biofungicides continue to be evaluated and over 30 materials have been tested either in the field or under greenhouse conditions. Thus far, none of the materials are efficacious at reducing downy mildew to commercially acceptable levels.

A bioassay has been developed to effectively test the effect of metalaxyl seed treatments on downy mildew disease control over time. US and EU treatment rates also were compared. Thus far, no isolates evaluated show any resistance to metalaxyl. The seed treatments show some efficacy after 30 days, particularly the seed treated at the higher EU rates.

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 known 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 2015 through March 2016, 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 against 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. A large set of commercial germplasm and PI's were screened against the newly nominated race 16 and UA1014.

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 bioassay. Several newer biofungicides have been evaluated this past year.

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.

Greenhouse bioassays were conducted to evaluate the effect of metalaxyl seed treatments and drench applications for downy mildew and establish a protocol to evaluate if isolates of the downy mildew pathogen are resistant to metalaxyl.

Results and Discussion

Objectives 1 and 2.

A total of 65 isolates were recovered and evaluated this past year. The majority of isolates examined were identified as 14, 16, or the UA1014 type. The disease reactions of the 16 reported races and UA1014 are shown in Table 1.

Table 1. Disease reactions of the 16 known races of the spinach downy mildew pathogen on the standard set of differential spinach lines.

Differential cultivar	Races of *Peronospora farinosa* f. sp. *spinaciae*																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	1014
Viroflay	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Resistoflay	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Califlay	-	+	-	+	-	+	+	-	-	+	-	-	+	-	+	-	+
Clermont	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+
Campania	-	-	-	-	-	+	-	+	+	+	-	+	+/-	+	-	+/-	+
Boeing[a]	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	-	+
Lion	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
Lazio	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+
Whale	-	-	-	(-)	-	(-)	(-)	-	-	+	-	+	+	-	+	-	+
Pigeon	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
Caladonia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
Meerkat	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ = 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. The reaction type of UA1014APLP type is shown (Table 2). The UA1014 type isolate has now been identified in 2014, 2015, and 2016 from CA, AZ, and TX on 10 different cultivars (Table 3).

Table 2. Disease reactions of the UA1014APLP type 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	+	+

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

Table 3. List of DM isolates, the host and geographic origin, and dates collected, that are the UA1014 type.

Isolate	Host Cultivar	Origin	Date Received	Result
UA1014A	Coati	Yuma, AZ	3/3/2014	1014 type
UA1914E	Silverwhale	King City, CA	5/6/2014	1014 type
UA3414G	Platypus	Salinas, CA	8/22/2014	1014 type
UA2015-13	Rigel	King City, CA	3/25/2015	1014 type
UA2015-16B	Platypus	King City, CA	4/15/2015	1014 type
UA2015-34A	Carerra	San Lucas, CA	8/21/2015	1014 type
UA2016-07	Siena	Uvalde, TX	2/20/2016	1014 type
UA2016-08C	Silverwhale	Yuma, AZ	2/25/2016	1014 type
UA2016-11A	Sp966	Yuma, AZ	3/15/2016	1014 type
UA2016-14C	Goldeneye	Wellton, AZ	4/5/2016	1014 type

Relating to Objective 1, a temperature controlled growth room was constructed in 2015 and put into operation in 2016 at the UC Cooperative Extension facility in Salinas. Based on the design of the UC Davis growth room for lettuce downy mildew research (Michelmore program), this facility will enable more precise control of temperature and humidity conditions during screening of spinach samples for downy mildew race identification.

Biofungicides (Objective 3)

We continue to evaluate biofungicides as they become available, since there are few acceptable options for downy mildew control of spinach under organic production conditions. Several recommended materials, including SporeControl, MildewControl, Zonix and Global1 were assessed under greenhouse conditions. These materials did not show any downy mildew control under these conditions. A newly acquired material, Vesta is currently being evaluated.

Seed Treatments (Objective 4)

A bioassay has been developed to test the effect of metalaxyl seed treatments on downy mildew disease control over time. Seed treatments included untreated seed and seed treated with either the US or EU rates. Seed treated in the EU have higher rates. In a rapid inoculation assay of 1-week-old plants, metalaxyl seed treatment was highly effective in reducing incidence of downy mildew indicating that this assay can readily be used to evaluate isolates for resistance to this widely used conventional fungicide (Fig. 1). Thus far, no isolates evaluated show any resistance to metalaxyl. The seed treatments were highly effective in reducing downy mildew and even showed some efficacy after 30 days, particularly the seed treated at the higher EU rates. (Fig. 2, Table 4).

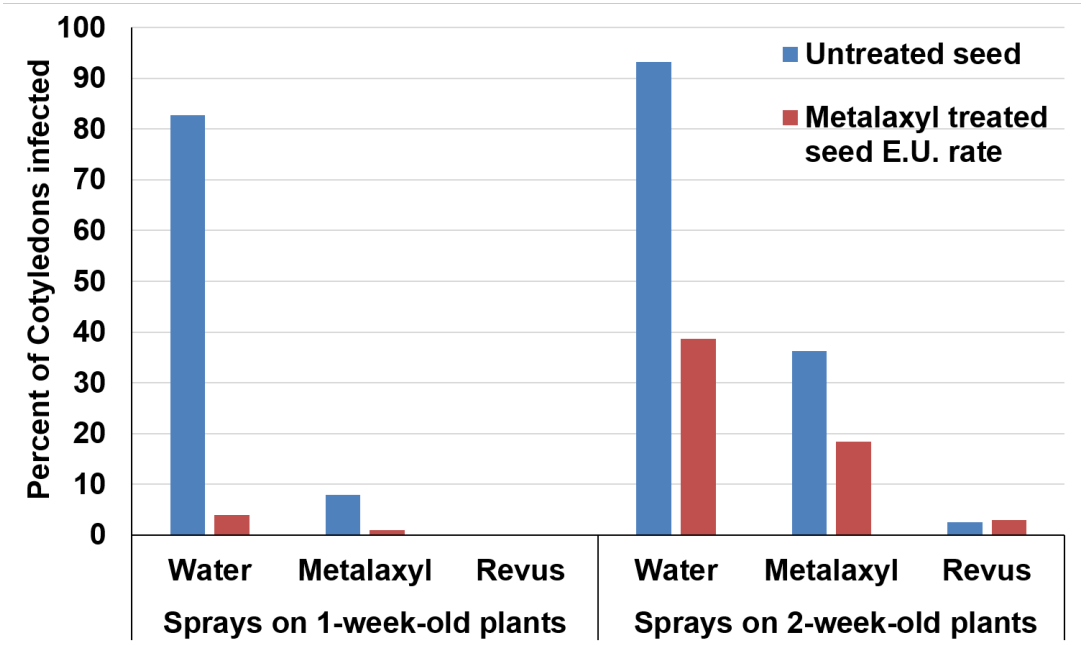

Figure 1. Downy mildew disease incidence on treated and untreated seed. Revus, a foliar applied fungicide was used as a control.

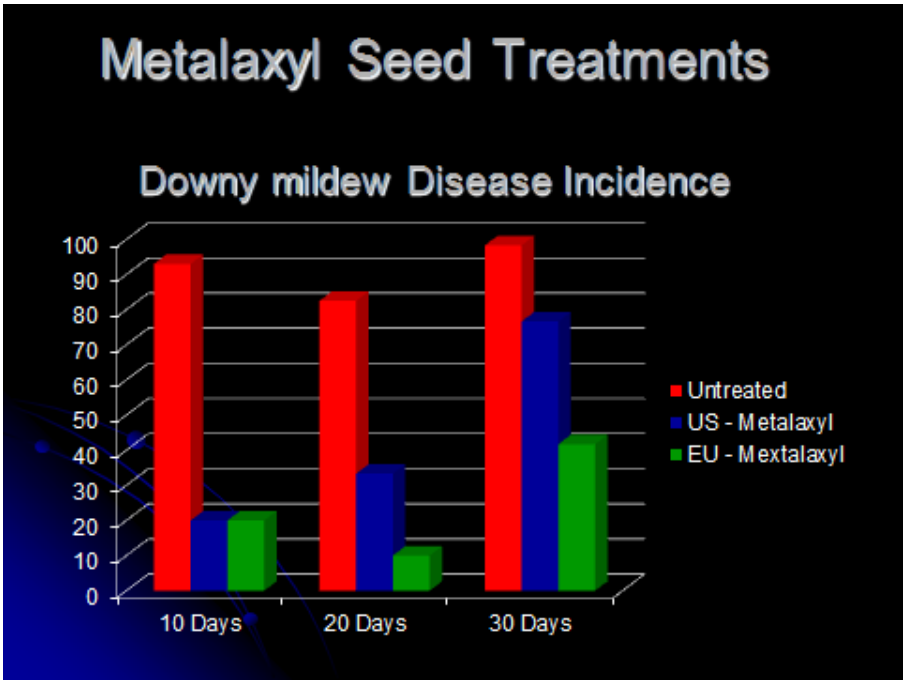

Figure 2. Disease incidence of downy mildew on 10, 20, and 30 day-old inoculated plants.

Table 4. Disease severity of seed pot-test result from 10 days, 20 days, 30 days plant.

Rate	Test 1			Test 2		
	10 days	20 days	30 days	10 days	20 days	30 days
Untreated	14.2	26.7	65.4	23.3	29.8	69.2
U.S. rate	0.8	2.5	27.5	1.7	15.8	36.7
E.U. rate	2.9	0.8	6.7	0.0	1.7	8.8

Disease severity was rated on a scale of 0-4, where 0 = no disease; 1 = 1-25% of the leaf are with symptoms; 2 = 26-50%; 3 = 51-75%; and 4 = >75%.

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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 in the past two years:

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.

Correll, J. C., and Feng, C. 2014. First report of *Peronospora farinosa* f. sp. *spinaciae* causing downy mildew on spinach in Egypt. Plant Disease 98:994.

Koike, S. T., and Correll, J. C. 2014. Race 15: new spinach downy mildew race confirmed. September 1. Salinas Valley Agriculture ANR blog. http://ucanr.edu/blogs/SalinasValleyAgriculture/index.cfm

Koike, S. T., Feng, C., Correll, J. C. 2015. Powdery mildew, caused by *Leveillula taurica*, on spinach in California. Plant Disease 99:555.

Feng, C., Bluhm, B. H., and Correll, J. C. 2015. Construction of a spinach bacterial artificial chromosome (BAC) library as a resource for gene identification and marker development. Plant Molecular Biology Reporter: DOI 10.1007/s11105-015-0891-9.

Qian, W., Feng, C., Zhang, H., Wei, L., Xu, D., Correll, J. C., Xu, Z. 2016. First report of race diversity in the spinach downy mildew pathogen, *Peronospora effusa* in China. Plant Disease 100: (in press).

Lyon, R., Correll, J. C., Feng, C., Bluhm, B., Shrestha, S., Shi, A., Lamour, K. 2016. Population structure of *Peronospora effusa* in the Southwestern United States. Plos One. DOI: 10.1371/journal.pone.0148385.

Koike, S. T., and Correll, J. C. 2016. Race 16: new spinach downy mildew race confirmed. March 2016. Salinas Valley Agriculture ANR blog. http://ucanr.edu/blogs/SalinasValleyAgriculture/index.cfm

Shi, A., Mou, B., Correll, J. C. 2016. Association analysis of resistance to Verticillium wilt in spinach. Journal of Phytopathology (in press).

Ma, J., Shi, A., Mou, B., Evans, M., Clark, J., Motes, D., Correll, J. C., Xiong, H., Qin, J., Chitwood, Y. and Weng, Y. 2016. Association mapping of leaf traits in spinach (*Spinacia oleracea* L.). Plant Breeding (submitted).

Shi, A., Mou, B., Correll, J. C., Koike, S. T., Motes, D., Qin, J., Weng, Y., and Yang, W. 2016. Association analysis of Stemphylium leaf spot resistance in spinach. American Journal of Plant Sciences (in press).

Correll, J.C., Matheron. M. E., Koike, S. T., Porchas, M., Pavel, J., and Feng, C. 2016. Evaluation of biofungicides and conventional fungicides for management of downy mildew on spinach. Plant Disease Management Reports (submitted).

Correll, J.C., Matheron. M. E., Koike, S. T., Saito, K., M., Pavel, J., and Feng, C. 2016. Evaluation of spinach varieties for downy mildew resistance. Plant Disease Management Reports (submitted).

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