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

Spinach downy mildew disease pressure was high again in the 2016-2017 season in both the desert of California/ Arizona and the central coast of California. A new race, race 16, was designated in 2016 and a number of novel races which are able to infect many of the race 1-15 and race 1-16 resistant cultivars were identified and are currently being further evaluated. Several isolates are also in a ringtest with the International Working Group on Peronospora and could potentially be designated as new races depending on the outcome of the tests in 2017. Race 16 and deviating isolate UA1014 predominated in samples collected in 2016. Two field trials, to evaluate cultivar reactions to downy mildew, were conducted. One trial was in the Salinas Valley in Sept-Oct. 2016 and one in the Yuma Valley in Jan.-Feb. 2017. Although downy mildew disease pressure in the Salinas Valley trial was high, some technical problems due to water issues resulted in poor stands making it difficult to get good disease ratings on the 70 cultivars evaluated. However, the trial in Yuma had ideal disease pressure and resulted in an excellent evaluation of downy mildew resistance among the 70 cultivars evaluated. These two trials are also planned for the coming season and we anticipate better results for the Salinas Valley trial. In addition, a range of conventional and biofungicide trials were evaluated in numerous greenhouse assays in both the greenhouse and the field in Yuma, AZ. We continue to evaluate new biofungicides to identify any that have efficacy. Several studies have examined Ridomil Gold (metalaxyl/mefenoxam) and Orondis (Oxathiapiprolin) to delay or prevent downy mildew development on young seedlings. Ridomil Gold significantly reduced disease development and Orondis prevented disease development. However, Orondis is not registered as a seed treatment. In addition, although not a specific objective supported by the CLGRB, a standardized test was

developed to evaluate oospores of the downy mildew pathogen on seed. The results of all of the studies outlined in the abstract are included in the Table and figure formats below.

Results and Discussion

Objectives 1, 2 and 5

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. Screen spinach germplasm from plant introduction collections (PIs), advanced breeding lines, and newer (especially race 1-15 resistant) commercially released material for resistance to various contemporary races of downy mildew.

5. Establish sentinel plots at the USDA field in Salinas to evaluate variety performance based on naturally occurring downy mildew pressure.

Over 170 inoculations were conducted up through March 2017 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 (race 16) or UA1014. The UA2015-19B 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 2017, 16 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 and 1-16 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 is being re-evaluated in a ringtest by the IWGP in the summer of 2017 but has not been nominated as a novel race type at this time. Several additional isolates recovered from race 1-16 resistant material are currently being evaluated in race tests.

Table 1. Disease reactions of the 16 known races of the downy mildew pathogen on 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[#]	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	-	+
Lion	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
Lazio	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+
Whale	-	-	-	(-)	-	(-)	(-)	-	-	+	-	+	+	-	+	-	+
Pigeon	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
Caladonia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
Meerkat	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Hydrus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2 Disease responses of spinach differential cultivars to races of *Peronospora farinosa f. sp. spinaciae* (*Pfs* 15 and *Pfs 16*) and eight novel isolates.

Cultivar	Parental Resistance Male	Female	*Pfs* 15	*Pfs* 16	UA1014	UA1414	UA201502	UA201511	UA201550	UA201621	UA201707A	UA201720B
Viroflay					+	+	+	+	+	+	+	+
Resistoflay	*RPF*5		+	+	+	+	+	+	+	+	+	-
Califlay		*RPF*3	+	-	+	+	+	-	-	+	-	-
Clermont	*RPF*4	*RPF*5	-	+	+	+	+	-	+	-	+	+
Campania	*RPF*6	*RPF*4	-	-	+	-	-	-	+	-	+	+
Boeing	*RPF*1	*RPF*5	-	-	+	-	-	-	+	-	+	-
Lion	*RPF*1	*RPF*3	-	-	+	-	-	-	-	-	-	-
Lazio	*RPF*2	*RPF*4	-	+	+	-	+	-	-	-	+	+
Whale	*RPF*3		+	-	+	+	+	-	-	+	-	-
Tarpy	*RPF*7		nt	-	+	nt	-	nt	+	-	+	-
Pigeon	*RPF*2	*RPF*9	-	+	(+)	-	+	+	-	+	+	+
Caladonia	*RPF*3	*RPF*9	+	-	(+)	+	+	-	-	+	-	-
Meerkat	*RPF*2	*RPF10*	-	+	(+)	-	+	+	-	+	+	-

+ = A plant showing chlorosis and sporulation on cotyledons, true leaves, or both was classified as susceptible. A spinach line was classified as susceptible if more than 85% of the plants were susceptible. Typically > 95% of the plants in a susceptible line show symptoms unless the line is segregating for resistance.

- = A plant without chlorosis or sporulation was rated as resistant.

(+) = Symptoms and sporulation were observed on cotyledons only and not on true leaves.

nt = not tested

Table 3. The disease reactions of 30 contemporary spinach cultivars to races *Pfs 16* of *Peronospora farinosa f. sp. spinaciae* and four novel isolates (UA1014, UA201621, UA201702A, and UA201707A).

Genotype	*Pfs* 16	UA1014	UA201621A	UA201702A	UA201707A
Sp966	-	(+)	-	-	+
Sp967	-	(+)	-	-	+
SV2146VB	-	(+)	-	−	+
Goldeneye	-	(+)	+	+	−
PV1206	-	(+)	+	+	−
Antalia	+	(+)	+	+	−
Hydrus	-	-/(+)*	-	−	−
Pegasum	+	-/(+)*	-	+	+
51-348	+	-/(+)*	+	+	+
51-351	-	-/(+)*	+	+	−
Caladonia	-	(+)	+	+	−
Coati	+	(+)	+	+	+
E03D.0579	-	(+)	+	+	−
Mandril	+	(+)	+	+	+
Platypus	+	(+)	+	+	+
Plover	+	(+)	+	+	+
PV-1053	-	(+)	+	+	−
Scorpius	-	(+)	+	+	-
Woodpecker	+	(+)	+	+	+
SSR-SP-29	-	+	+	+	−
Antalia	+	(+)	+	+	−
Cealum	+	(+)	+	+	−
Carrera	-	(+)	+	+	−
Mandril	+	(+)	+	+	+
Shelby	-	(+)	-	−	+
Tasman	-	+	+	+	−
Virgo	+	(+)	+	+	−
Volans	+	(+)	+	+	−
PV1202	+	(+)	-	+	+
Wombat	+	(+)	+	+	−

+ = A plant showing chlorosis and sporulation on cotyledons, true leaves, or both was classified as susceptible. A spinach line was classified as susceptible if more than 85% of the plants were susceptible. Typically > 95% of the plants in a susceptible line show symptoms unless the line is segregating for resistance.

- = A plant without chlorosis or sporulation was rated as resistant.

(+) = Symptoms and sporulation were observed on cotyledons only and not on true leaves.

*For the lines indicated, the results varied for individual tests.

Fig. 1. Frequency of race or novel strain of the downy mildew pathogen recovered by year.

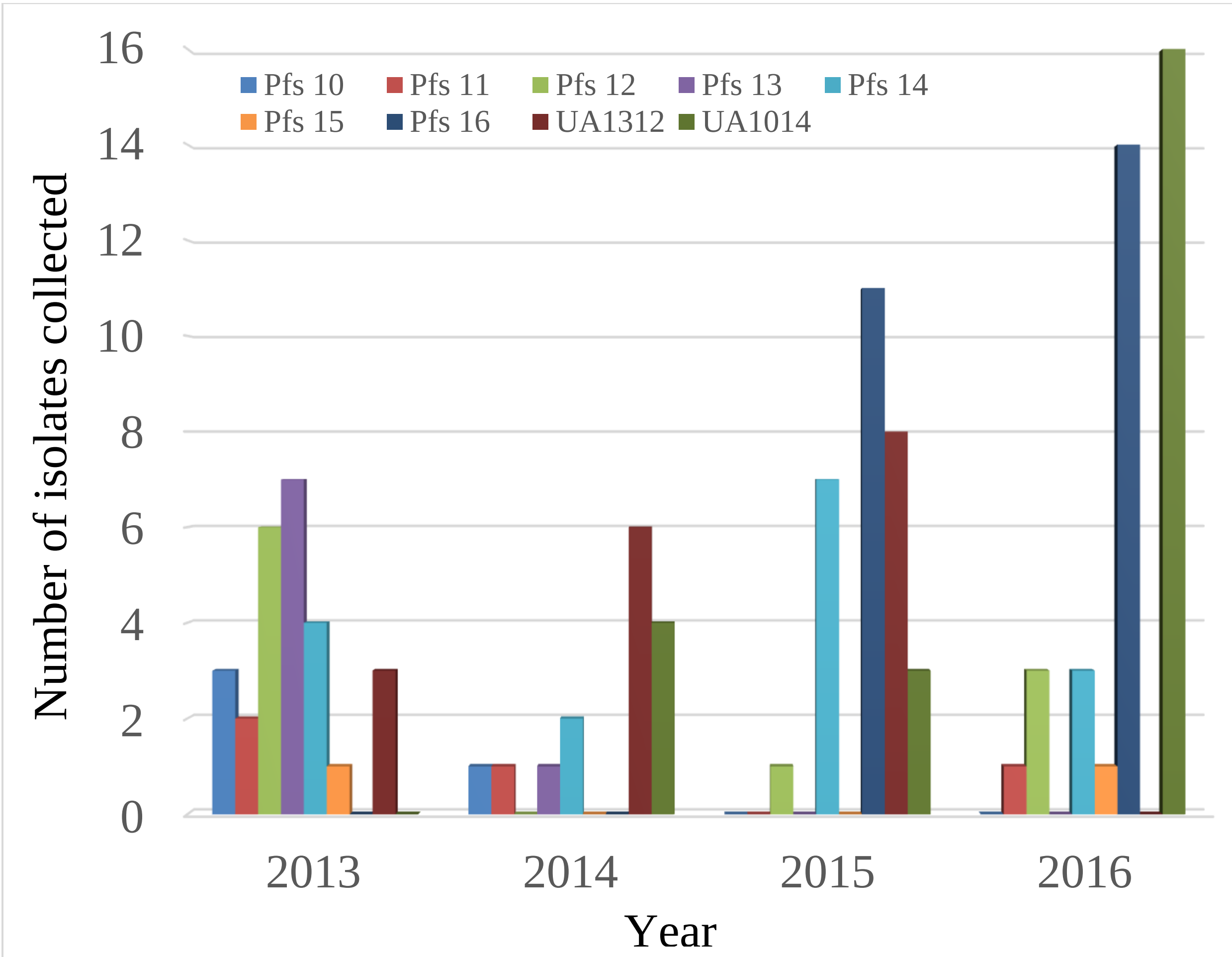


Table 4. Cultivar evaluations in Yuma, AZ, 2017. Based on the genetic background of the cultivars and testing isolates from the trial, pathogen races 14 and 16, and novel isolates UA1014, UA2016-21A and UA2017-02A were present in the naturally infested field plots.

Variety	Disease incidence[z]		Disease severity[y]		Variety	Disease incidence		Disease severity	
SV1714VC	0.91	a[x]	60.0	a	SV-2 7989	0.16	i-p	3.9	m-q
Responder	0.72	ab	45.2	bc	Cepheus	0.16	i-p	3.7	m-q
SV2157VB	0.72	ab	50.7	ab	Rembour	0.15	j-p	7.8	i-q
SV3580VC	0.69	a-c	39.7	cd	Yukon	0.13	j-p	2.5	n-q
SV2146VB	0.68	a-c	37.3	cd	PV-1292	0.12	k-p	7.8	i-q
Molokai	0.67	a-c	38.6	cd	Yuma1	0.11	k-p	1.8	n-q
Revere	0.64	b-d	34.2	de	Yuma2	0.11	k-p	1.4	n-q
Viroflay	0.58	b-e	25.0	ef	SV-5 8414	0.11	k-p	1.5	n-q
Renegade	0.49	b-f	24.4	e-g	Goldeneye	0.11	k-p	3.0	n-q
PV-1190/Apache	0.48	b-f	10.6	h-p	Mandolin	0.11	k-p	1.9	n-q
Cello	0.47	c-g	14.8	f-k	SV-1 0282	0.10	k-p	1.3	n-q
Monza	0.45	c-h	16.9	f-j	Silverwhale	0.09	k-p	1.3	n-q
PV-1194	0.41	d-i	17.0	f-j	Sioux	0.09	k-p	1.3	n-q
Meerkat	0.41	d-i	14.7	f-l	Galah	0.09	k-p	1.3	n-q
Carrera	0.38	e-j	10.2	h-q	Tasman	0.09	k-p	2.3	n-q
Scorpius	0.38	e-j	17.8	f-i	SV-3 0421	0.08	l-p	1.4	n-q
Caladonia	0.37	e-j	19.3	f-h	PV-1451	0.08	l-p	1.1	n-q
Camaro	0.33	e-k	10.8	h-o	Finwhale	0.06	m-p	1.1	n-q
Platypus	0.31	f-l	14.2	g-m	PV-1445	0.05	m-p	0.8	n-q
SV-4 8155	0.30	f-m	10.1	h-q	Remake	0.05	m-p	0.8	n-q
SV-6 5044	0.27	f-n	8.23	i-q	Antalia	0.04	n-p	0.8	n-q
Regiment	0.26	f-o	5.15	k-q	Hydrus	0.04	n-p	0.5	o-q
Acadia	0.25	f-o	7.69	i-q	PV-1290	0.04	n-p	0.5	o-q
Spiros	0.24	f-p	8.09	i-q	Volans	0.03	n-p	0.4	o-q
Piano	0.24	f-p	6.79	j-q	Serpens	0.03	n-p	0.3	o-q
Boeing	0.22	g-p	6.80	j-q	Woodpecker	0.02	n-p	0.3	o-q
Pegasum	0.22	g-p	4.31	k-q	Cursa	0.02	op	0.3	o-q
Reflect	0.22	g-p	6.07	k-q	PV-1444	0.02	op	0.3	o-q
Callisto	0.21	h-p	11.4	h-n	PV-1206	0.02	op	0.2	o-q
Shelby	0.21	h-p	4.16	l-q	PV-1415	0.02	op	0.2	o-q
Sparrow	0.21	h-p	5.23	k-q	PV-1446	0.01	op	0.2	o-q
PV-1202/Sioux	0.18	i-p	5.88	k-q	El Prado	0.01	op	0.4	o-q
Yukon	0.17	i-p	7.79	i-q	Canapus	0.00	p	0.1	q
Carmel	0.17	i-p	3.71	m-q	Virgo	0.00	p	0.1	q
Andromeda	0.17	i-p	3.1	n-q	Alcor	0.00	p	0.1	q

[z] Disease incidence (DI) was calculated as follows: DI = (B+C+D+E) / (A+B+C+D+E), where A, B, C, D, and E = # of leaves with a score of 0, 1, 2, 3, or 4, respectively.

[y] Disease severity (DS) was calculated using the midpoint of each category, for example, the midpoint for a rating of 1 (1-25%) was 12.5. DS = [(A*0) + (B*12.5) + (C*37.5) + (D*62.5) + (E*87.5)] /(A+B+C+D+E).

[x] Analysis of variance (ANOVA) was performed using R programming language on the two traits of disease severity and arcsine transformed disease incidence, the means of DS and DI of these varieties were compared using the least significant difference test (p<0.05) . Variety means with the same letter are not significantly different as determined by Fisher's LSD test (P=0.05). The LSD for disease severity was 10.6 and for disease incidence was 0.25.

Objective 3

Examine organic products for their effectiveness in reducing downy mildew on spinach under greenhouse and field conditions.

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. New materials continue to be evaluated. In cooperation with Dr. Mike Matheron, a field trial was conducted with conventional and biofungicide materials. The results are presented in Table 5.

Many of the treatments provided a statistically significant reduction of disease compared to nontreated plants; however, four treatments provided exceptional disease control, reducing the percentage of infected leaves to about 2% compared to a level of 60% in nontreated plots. Downy mildew was first observed in plots on 10 Feb, nine days after the first application of foliar treatments; therefore, few if any infections likely had occurred by the first general foliar application date of 1 Feb. Phytotoxicity symptoms were not noted for any treatments.

Table 5. Field trial in Yuma, Az to evaluate conventional and biofungicide materials for downy mildew control.

Treatment and rate of product/A	Days after first application[z]	Percent infected leaves[y]
Ridomil Gold SL 20.0 fl oz + Quadris 2.08SC 10.6 fl oz	0	1.8
Presidio 4SC 4.0 fl oz + Forum 6.0 fl oz	7	
Prophyt 6.64SL 4.0 fl oz	20	
Presidio 4SC 4.0 fl oz + Prophyt 6.64SL 4.0 fl oz	28	
Ridomil Gold SL 20.0 fl oz + Quadris 2.08SC 10.6 fl oz	0	1.8
Actigard 50WG 0.75 oz	7, 20	
A-21591 5.5 fl oz	28	
Ranman 2.75 fl oz	7, 28	2.2
STK73 21.0 fl oz	20	
Ridomil Gold SL 20.0 fl oz + Quadris 2.08SC 10.6 fl oz	0	2.2
Actigard 50WG 0.75 oz	7	
A-21591 5.5 fl oz	20	
Actigard 50WG 0.75 oz	28	
Ridomil Gold SL 20.0 fl oz + Quadris 2.08SC 10.6 fl oz	0	17.0
A-21591 5.5 fl oz	7	
Actigard 50WG 0.75 oz	20, 28	
Ranman 2.75 fl oz + Silwet L-77 2.0 fl oz	7, 20, 28	20.0
Zampro 14.0 fl oz	0, 7, 20	32.0
Ranman 2.75 fl oz	7, 28	40.0
Timorex Gold 28.0 fl oz	20	
Ranman 2.75 fl oz	7, 28	40.0
STK73 28.0 fl oz	20	
Timorex Gold 6.4 fl oz	7, 20, 28	40.0
Zampro 14.0 fl oz	7, 20	40.0
Oxidate 2.0 160.0 fl oz	7, 20, 28	40.0
Ranman 2.75 fl oz	7, 28	40.0
Timorex Gold 14.0 fl oz	20	
Mildicut 22.0 fl oz	7, 20, 28	40.0
Mildicut 33.0 fl oz	7, 20, 28	40.0
GC Pro 3.0 lb	7, 20, 28	48.0
Timorex Gold 64.0 fl oz	7, 20, 28	48.0
ISO NPK 8.0 fl oz	7, 20, 28	48.0
GC Pro 1.5 lb	7, 20, 28	48.0
Procidic 15.0 fl oz	0, 7, 20, 28	52.0
Oxiphos 5.0 qt	7, 20, 28	52.0
Timorex Gold 32.0 fl oz	7, 20, 28	56.0
GWN-10580 8.0 qt	7, 20, 28	56.0
GC Pro 0.5 lb	7, 20, 28	60.0
Procidic 15.0 fl oz	0, 7, 20, 28	60.0
DUOLIF 19.2 oz	7, 20, 28	60.0
GWN-10580 2.0 qt	7, 20, 28	60.0
Vesta 15.0 gal + Blue Cal 60 3.0 pt	0	60.0
Vesta 15.0 gal + Blue Cal 60 2.0 pt	5, 14	60.0
Nontreated control	-------	60.0
LSD (P = 0.05)[x]		7.6

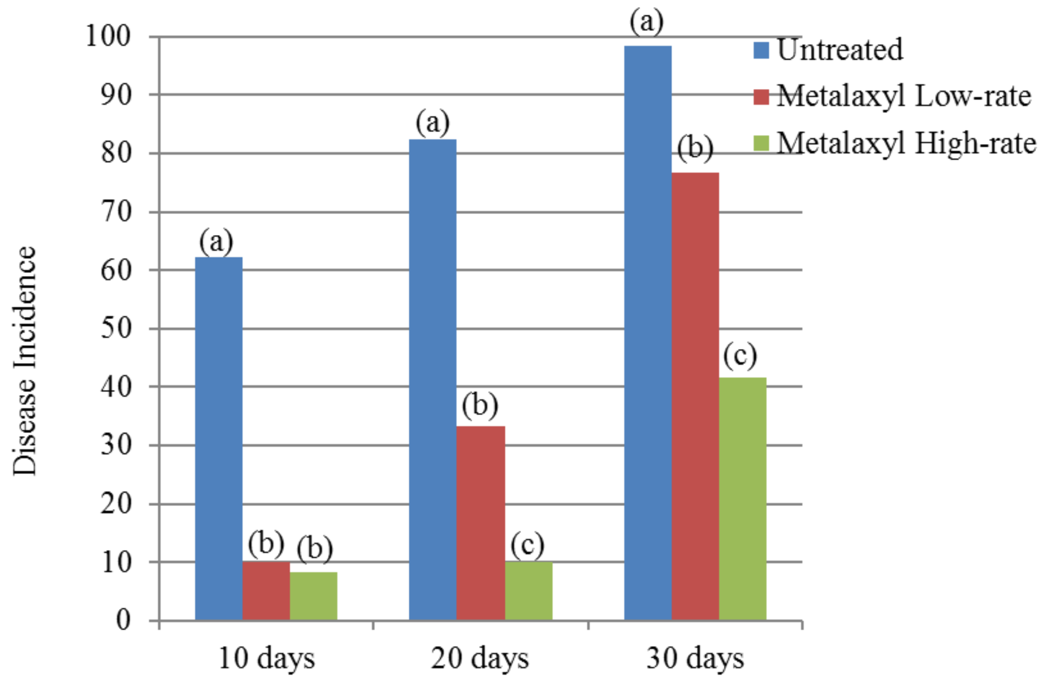
[z] Application date for at emergence treatments was 25 Jan and other foliar treatments were applied 30 Jan; 1, 8, 13, and 21 Feb, depending on the treatment.

[y] Disease severity was assessed 23-24 Feb by determining the percentage of infected leaves present within three 1-ft^2 areas within each of the five replicate plots per treatment.

[x] Least Significant Difference at P = 0.05. Values differing by more than the least significant difference are significantly different from each other according to Fisher's Protected LSD test.

Fungicide Seed Treatments

We continue to evaluate registered (Ridomil Gold) and unregistered (Orondis) fungicides for the potential to reduce or delay infection on young spinach seedlings. Although some variation in efficacy has been observed with Ridomil Gold (metalaxyl/mefenoxam), it is still effective at reducing downy mildew on seedlings when plants are challenged up to 30 days after planting. Although Orondis is not registered as a seed treatment, it was exceptionally effective at reducing downy mildew when seedlings were challenged 30 days after planting.



(B)

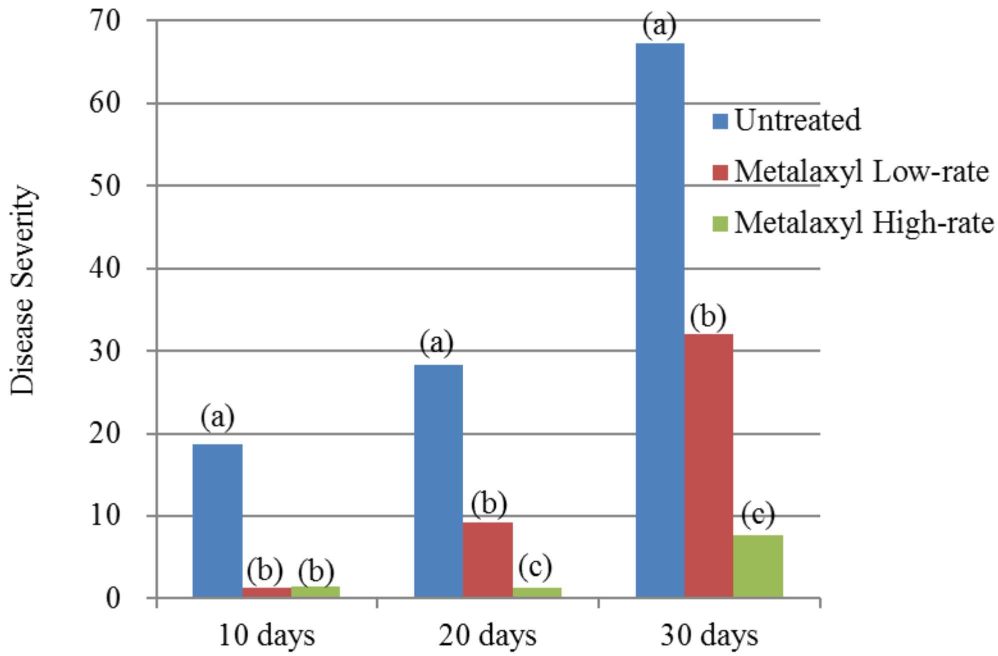


Figure 2. Ridomil Gold (metalaxyl/mefenoxam) treated and untreated seed plants. Plants were inoculated with *Pfs* race 16 (UA19B) at 10, 20, and 30 days. Disease incidence (A) and disease severity (B) were analyzed by disease scoring formula. The different characters in the chart indicate results are significantly different at P=0.05.

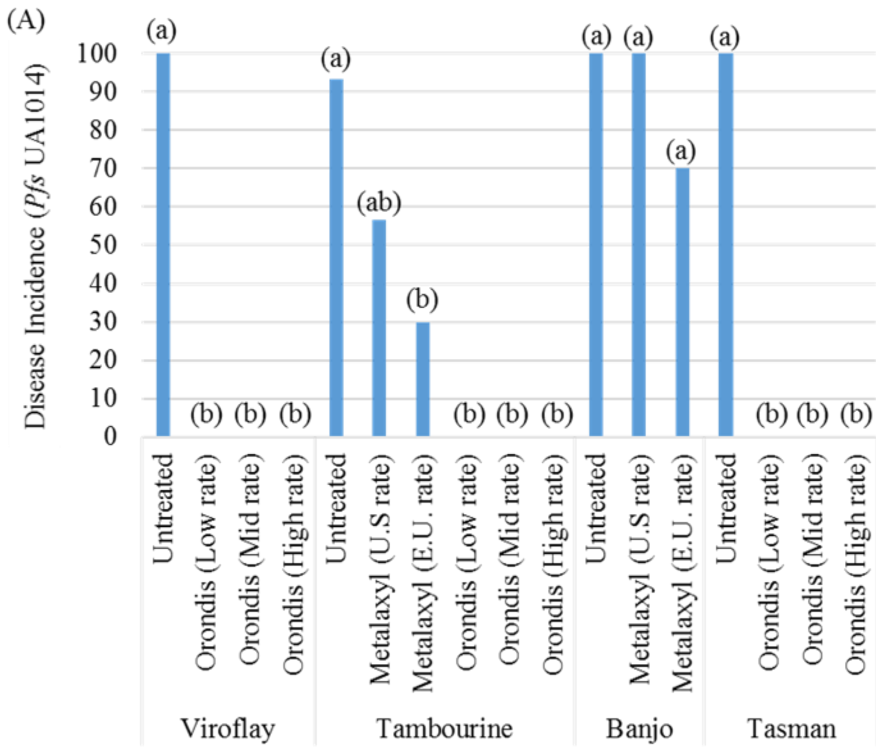


Figure 3. Disease incidence of spinach plants grown from fungicide treated seed and inoculated with Pfs UA1014. Treatments followed by the same letter are not significantly different at P=0.05.

Research papers associated with spinach efforts from our team from the past year:

Qian, W., Feng, C., Zhang, H., Wei, L., Xu, D., Correll, J. C. 2, Xu, Z. 2016. First report of race diversity in the spinach downy mildew pathogen, Peronospora effusa in China. Plant Disease 100:1248.

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

Shi, A., Mou, B., Correll, J. C. 2016. Association analysis of resistance to Verticillium wilt in spinach. Australia J. of Plant Science 10(8):1188-1196.

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 35:399–404 (doi:10.1111/pbr.12369).

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 7:1600-1611.

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 10:V106.

Correll, J.C., Matheron. M. E., Koike, S. T., Porchas, M., Pavel, J., and Feng, C. 2016. Evaluation of spinach varieties for downy mildew resistance. Plant Disease Management Reports 10:V107.

Shi, A., B. Mou, J. C. Correll. 2016. Association analysis for oxalate concentration in spinach. Euphytica (DOI: 10.1007/s10681-016-1740-0).

Correll, J. C. Feng, C. and Liu, B. 2017. First report of white rust (Albugo occidentalis) of spinach in Mexico. Plant Disease 101: (doi: http://dx.doi.org/10.1094/PDIS-06-16-0905-PDN).

Feng, C. Katsunori Saito, Katherine Kammeijer, Stacy J. Mauzey, Steven Koike, and James C. Correll. 2017. New races and novel strains of spinach downy mildew pathogen Peronospora effusa. Plant Disease 101: (submitted).

M.E. Matheron, J. C. Correll, M. Porchas, C. Feng. 2017. Assessment of fungicides for managing downy mildew of spinach, 2017. Plant Disease Management Reports 11:V108.

Correll, J. C., C. Feng, M.E. Matheron, and M. Porchas, and S. T. Koike. 2017. Evaluation of spinach varieties for downy mildew resistance. Plant Disease Management Reports 11:V108.
Liu, Bo, Chunda Feng, Michael E. Matheron and James C. Correll. 2017. Characterization of foliar web blight of spinach in the desert southwest caused by Pythium aphanidermatum. Plant Disease 101: (submitted).

Feng, C., Lamour, K. H., Bluhm, B. H., Sharma, S., Shrestha,S. Dhillon, B. D. S., and Correll, J. C. 2017. Genome sequences of Peronospora effusa. Genome Announcements (submitted).

Shi, A., Jun Qin, Beiquan Mou, J.C. Correll, Yuejin Weng, David Brenne, Chunda Feng, Dennis Motes, Wei Yang, Lingdi Dong, Gehendra Bhattarai, and Waltram Ravelombola. 2017. Genetic diversity and population structure analysis of spinach by single-nucleotide polymorphisms identified through genotyping-by-sequencing. Theoretical and Applied Genetics (submitted).