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

Spinach downy mildew disease pressure was relatively low in the 2017 and the early part of the 2018 season. A new race, race 17, was reported in 2018 after numerous ringtests with the International Working Group on Peronospora (IWGP). The race 17 was similar to what growers and the industry were calling UA1014 that was identified previously. A total of 75 mildew isolates were collected during the past reporting period and evaluated for their race identity. Races 8, 14, 16, 17, and the novel isolate UA2016-21A, were the most common. A race 5 and a race 10 were recovered from Utah, and Washington state, respectively. Numerous isolates were obtained from the East Coast and examined. A wide range of isolates were identified as either races 12, 15 or a novel isolate UA2017-20B. The novel isolate (UA2017-20B) was recovered from four different locations on the East Coast in 2017 and 2018.

Two large-scale field trials were conducted to evaluate cultivar reactions to downy mildew. One trial was in the Salinas Valley in Sept-Oct. 2017 and one in the Yuma Valley in Jan.-Feb. 2018. Disease pressure was high at both locations and a wide range in reactions were observed among the cultivars evaluated. An industry-wide field day was held in Salinas on Oct 11, 2017 and in Yuma, AZ in Feb 21, 2018 with approximately 120 participants at each event. In addition, a range of conventional and biofungicide trails 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 additional efforts being pursued include the development and standardization of a test to quantify oospores on seed, the evaluation of factors that can reduce viability of oospores on seed, and crosses of various isolates to determine how oospores are formed in plants and on seed. We have also developed a detached soil-less leaf inoculation test to evaluate resistance to downy mildew disease. Recent greenhouse tests indicate that we can generate oospores in plants by doing inoculations with mixtures of downy mildew isolates. This development will be critical in evaluating the role of oospores in the epidemiology of the pathogen.

Results and Discussion

- 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.

- 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.

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

- Evaluate seed treatments of metalaxyl and oxathiapiprolin (Orondis) applications for the effectiveness in controlling downy mildew and for the longevity of control (this work was completed midway through 2017).

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

- Work with seed companies to establish protocol for evaluating seed infestation of the downy mildew pathogen.

A series of ringtests were performed by the IWGP and the group denominated race 17 in April 2018. http://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=26906 as a result of the efforts supported by the CLGRB through Correll and Koike's lab. The race 17 strain was what most growers in California and Arizona refer to as UA1014. (see details below).

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Denomination of Pfs: 17, a new race of downy mildew in spinach

Author: Jim Correll **Published on: April 16, 2018**

A new race of the downy mildew pathogen (*Peronospora farinosa* f. sp. *spinaciae* = *P. effusa*) on spinach was first identified in 2014 in Yuma, Arizona, U.S. This race was able to overcome the resistance of important spinach varieties. First, one isolate (UA1014), was characterized on a standard set of differential varieties. Subsequently, isolates with the same reaction pattern on these differential varieties have been found in numerous locations and each year since 2014. After careful evaluation of the significance of this development to the spinach industry, the International Working Group on *Peronospora* in spinach (IWGP) has denominated isolate US1602 as Pfs: 17. This isolate is available at Naktuinbouw in The Netherlands.

Race Pfs: 17 is able to infect the differentials NIL1-6, Whale, Pigeon, and Caladonia. Meerkat has intermediate resistance. The reaction of Meerkat can be variable in seedling tests. Hydrus is resistant.

The IWGP is continuously monitoring the appearance of strains of the pathogen that deviate in virulence from the known races. In this way the IWGP aims to promote a consistent and clear communication between public and private entities, such as the seed industry, growers, scientists, and other interested parties about all resistance-breaking races that are persistent enough to survive over several years, occur in a wide area, and cause a significant economic impact.

The IWGP is located in The Netherlands and is administered by Plantum NL. The IWGP consists of spinach seed company representatives (Pop Vriend, Monsanto, RijkZwaan, Bayer, Takii, Sakata, Bejo, Enza, Syngenta, and Advanseed) and Naktuinbouw, and is supported by research centers at the University of Arkansas. Spinach researchers over the world are invited to join the IWGP initiative and use the common host differential set to identify new isolates.

For more information on this subject you can contact Jim Correll (jcorrell@uark.edu), Diederik Smilde (d.smilde@naktuinbouw.nl), or the IWGP chairperson Jan de Visser (JandeVisser@popvriendseeds.nl).

The table below includes the disease reactions of race 17 on the International set of differential spinach cultivars.

Race	Differential											
	Viroflay	NIL 5	NIL 3	NIL 4	NIL 6	NIL 1	NIL 2	Whale	Pigeon	Caladonia	Meerkat	Hydrus
Pfs: 1	+	-	-	-	-	-	-	-	-	-	-	-
Pfs: 2	+	-	+	-	+	-	-	-	-	-	-	-
Pfs: 3	+	+	-	-	-	-	-	-	-	-	-	-
Pfs: 4	+	+	+	-	-	-	-	(-)	-	-	-	-
Pfs: 5	+	+	-	+	-	-	-	-	-	-	-	-
Pfs: 6	+	+	+	+	+	-	-	(-)	-	-	-	-
Pfs: 7	+	+	+	+	-	-	-	(-)	-	-	-	-
Pfs: 8	+	+	-	+	+	+	-	-	-	-	-	-
Pfs: 9	+	+	-	+	+	-	-	-	-	-	-	-
Pfs: 10	+	+	+	+	+	+	-	(-)	-	-	-	-
Pfs: 11	+	+	-	+	-	-	+	-	-	-	-	-
Pfs: 12	+	+	-	+	+	+	+	-	-	-	-	-
Pfs: 13	+	+	+	+	(-)	-	+	+	-	-	-	-
Pfs: 14	+	+	-	+	+	+	+	-	+	-	-	-
Pfs: 15	+	+	+	-	-	-	-	(-)	-	+	-	-
Pfs: 16	+	+	-	+	-	-	+	-	+	-	+	-
Pfs: 17	+	+	+	+	+	+	+	+	+	+	(-)	-

Over 150 inoculations were conducted up through March 2018 to examine the race diversity of downy mildew in California and Arizona. The majority of the isolates tested were identified as races 8, 14, 16, 17, and the novel isolate UA2016-21A, were the most common. A race 5 and a race 10 were recovered form Utah, and Washington state, respectively. Numerous isolates were obtained from the East Coast and examined. A wide range of isolates were identified as either races 12, 15 or a novel isolate UA2017-20B. The novel isolate (UA2017-20B) was recovered from four different locations on the East Coast in 2017 and 2018.

A list of novel isolates that appear to be unique in their disease reaction on differentials. Isolate UA2016-21A is being evaluated by the IWGP in a ringtest for race determination.

	UA201621A	UA201702A	UA201707A	UA201720B	UA201741B
Viroflay	+	+	+	+	+
Resistoflay	+	+	+	-	-
Califlay	+	+	-	-	-
Clermont	-	+	+	+	+
Campania	-	-	+	+	+
Lion	-	-	-	-	-
Lazio	-	+	+	+	+
PV1202	-	+	+	-	-
PV1206	+	+	-	-	-
PV1446	-	-	-	-	-
NIL1	-	-	+	+	-
NIL2	+	+	-	+	+
NIL3	+	+	-	-	-
NIL4	-	+	+	+	+
NIL5	+	+	+	-	-
NIL6	-	-	+	+	+
Tarpy	-	-	+	-	-
Pigeon	+	+	+	+	+
Meerkat	-	+	+	-	-
Hydrus	-	-	-	-	-
Caladonia	+	+	-	-	-

Evaluation of spinach varieties for downy mildew resistance, Monterey County, CA, 2017. Final disease evaluation was on October 18. 2017.

Cultivar	Disease incidence[z]		Cultivar	Disease incidence		Cultivar	Disease incidence	
Kookaburra	91.7	a[y]	Melville	45.0	h-o	Woodpecker	16.7	p-v
Corvair	91.7	a	SV3580VC	43.7	h-o	Goldeneye	15.0	q-v
SV1714VC	88.3	a-b	Magnetic	43.3	i-p	Finwhale	10.3	r-v
SV2157VB	86.7	a-c	Escalade	43.3	i-o	El Rio	10.0	r-v
Pacer	83.3	a-c	Midway	40.0	i-q	Galah	8.7	s-v
Viroflay	81.7	a-d	Hamilton	40.0	i-q	Cepheus	7.0	s-v
Yukon	75.0	a-e	Enclave	40.0	i-q	Cursa	2.7	t-v
Shelby	73.3	a-e	Silverwhale	38.3	j-r	Canapus	2.7	t-v
Carrera	71.7	b-f	Spiros	38.3	i-r	El Prado	2.0	u-v
Remake	70.0	c-h	Platypus	36.7	j-s	Alcor	2.0	u-v
Rembour	70.0	c-g	Tasman	36.7	j-r	Serpens	1.7	u-v
SV2994VC	68.3	c-h	Space	35.0	j-s	Volans	1.5	u-v
SV2146VB	63.3	d-i	Carmel	31.7	k-t	Virgo	1.0	u-v
Camaro	58.3	e-j	Renegade	30.0	l-v	PV 1444	0.7	u-v
Andromeda	56.7	e-k	Revere	30.0	l-u	PV 1452	0.3	u-v
Regiment	53.3	f-m	RZ#1	28.3	m-v	Antalia	0.3	u-v
Reflect	53.3	f-m	Hydrus	26.7	m-v	PV 1445	0.0	v
Responder	53.3	e-l	PV 1484	23.3	n-v	PV 1446	0.0	v
Meerkat	50.0	g-n	Parakeet	21.7	o-v	PV 1449	0.0	v
Java	46.7	g-n	Sioux	21.0	o-v	PV 1477	0.0	v

[z] Disease incidence (DI) was estimated based on visually inspecting a 1 x 1 m sq.. area in the center of each plot.
[y] Analysis of variance (ANOVA) was performed using R programming language on the arcsine transformed disease incidence, the means of 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 incidence (untransformed data) was 30.5%.

Evaluation of spinach varieties for downy mildew resistance, Yuma, AZ 2018. Final disease evaluation was on February 28, 2018.

Cultivar	Disease incidence[z]		Cultivar	Disease incidence		Cultivar	Disease incidence	
SV1714VC	100.0	a[y]	Camaro	10.7	lm	Canapus	0.0	m
SV2157VB	90.0	b	Goldeneye	10.0	lm	Cursa	0.0	m
Remake	86.7	bc	Rembour	10.0	lm	El_Prado	0.0	m
SV2146VB	86.7	bc	Midway	8.0	lm	El_Rio	0.0	m
Molokai	83.3	b-d	Tabit	5.7	m	Elund	0.0	m
Kookaburra	83.3	b-d	Antalia	4.7	m	Galah	0.0	m
SV3580VC	80.0	b-e	Virgo	4.7	m	Lorikeet	0.0	m
Renegade	70.0	c-f	Acadia	4.0	m	Meerkat	0.0	m
Regiment	70.0	d-f	RZ1	2.7	m	Menkar	0.0	m
Corvair	63.3	ef	Java	2.0	m	Monterey	0.0	m
Cello	60.0	e-g	Platypus	2.0	m	Nembus	0.0	m
Enclave	60.0	fg	Silverwhale	2.0	m	PV1444(Nevada)	0.0	m
Revere	60.0	fg	Tasman	1.7	m	PV1445(Colusa)	0.0	m
Pacer	57.3	fg	Parentie	1.7	m	PV1446(Kiowa)	0.0	m
Tundra	55.0	f-h	Magnetic	1.3	m	PV1452	0.0	m
Reflect	50.0	f-i	Cepheus	1.0	m	PV1477	0.0	m
Shelby	40.0	g-j	Space	1.0	m	PV1484	0.0	m
Carmel	37.3	g-j	Alcor	1.0	m	Serpens	0.0	m
Escalade	36.7	h-k	Finwhale	0.7	m	Sioux	0.0	m
Responder	36.7	i-k	Hamilton	0.3	m	Spiros	0.0	m
Yukon	30.0	j-l	Melville	0.3	m	Volans	0.0	m
SV2994VC	28.0	j-l	Parakeet	0.3	m	Whale	0.0	m
Hydrus	15.0	k-m	PV1449	0.3	m			
Carrera	11.7	lm	Woodpecker	0.3	m			

[z] Disease incidence (DI) was estimated based on visually inspecting a 1 x 1 m sq. area in the center of each plot.

[y] Analysis of variance (ANOVA) was performed using R programming language on the arcsine transformed disease incidence, the means of 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 incidence (untransformed data) was 25.1%.

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 in Jan. – Feb. 2018. The results are currently being fully analysed. One organic material that growers are using, and have frequently asked about, is LifeGard. This material has not been effective in reducing downy mildew in greenhouse bioassays and has only been minimally effective in slightly reducing diease incidence un field conditions.

Some of the treatments provided a statistically significant reduction of disease compared to nontreated plants; however, only conventional materials provided disease control, reducing the percentage of infected leaves to just trace infections compared to a level of 82% in nontreated plots. Downy mildew was first observed in plots on 15 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.

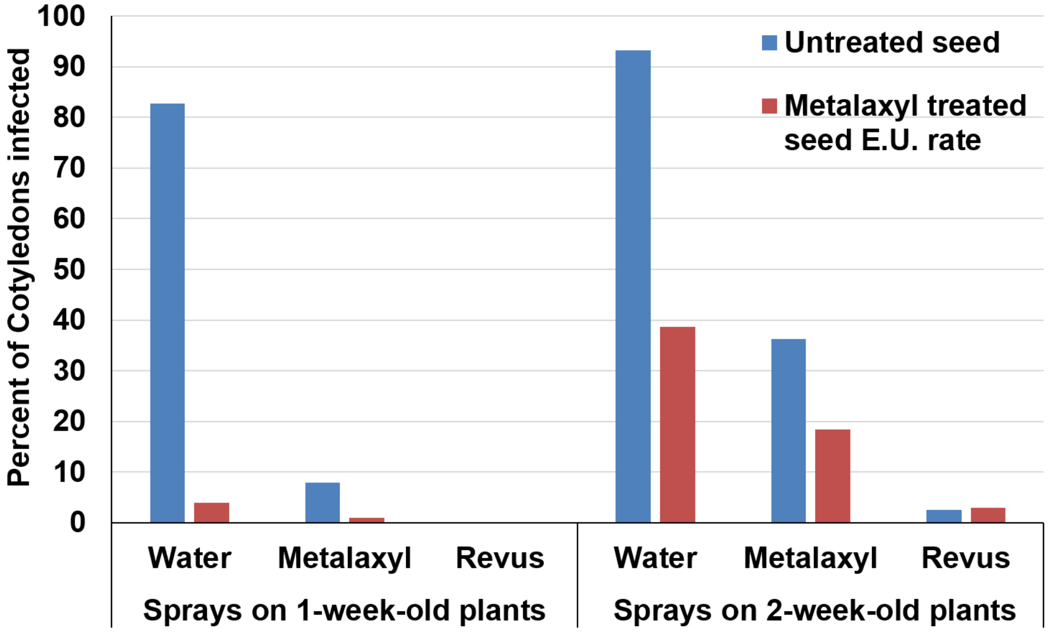
Field evaluation of conventional and organic materials in Yuma, AZ in 2018. The trial was conducted in cooperation with Dr. Mike Matheron.

TREATMENT	Treatment dates	Rate per acre	Mean Disease (Percentage)
Ridomil Gold 480SL	25-Jan	1.25 pt	0.0
+ Quadris 2.08SC		10.6 fl oz	
Actigard 50WG	31-Jan	0.75 oz	
A-21591	6-Feb	5.5 fl oz	
Actigard 50WG	19-Feb	0.75 oz	
Revus 250SC	27-Feb	8.0 fl oz	
Ridomil Gold 480SL	25-Jan	1.25 pt	0.0
+ Quadris 2.08SC		10.6 fl oz	
Actigard 50WG	31 Jan, 6 Feb	0.75 oz	
A-21591	19-Feb	5.5 fl oz	
Revus 250SC	27-Feb	8.0 fl oz	
Actigard 50WG	31-Jan	1.0 oz	0.0
Orondis Gold 200	6-Feb	4.8 fl oz	
+ Ridomil Gold 480SL		8.0 fl oz	
Revus 250SC	19-Feb	8.0 fl oz	
Zampro 4.38SL	27-Feb	14.0 fl oz	
Orondis Gold 200	31 Jan, 6, 19, 27 Feb	4.8 fl oz	0.0
+ Ridomil Gold 480SL		8.0 fl oz	
Actigard 50WG	31 Jan, 19 Feb	1.0 oz	10.0
Revus 250SC	6 and 27 Feb	8.0 fl oz	
Ridomil Gold 480SL	25-Jan	1.25 pt	12.5
+ Quadris 2.08SC		10.6 fl oz	
A-21591	31-Jan	5.5 fl oz	
Actigard 50WG	6 and 19 Feb	0.75 oz	
Revus 250SC	27-Feb	8.0 fl oz	
Revus 250SC	31 Jan, 6, 19, 27 Feb	8.0 fl oz	20.0
Actigard 50WG	31 Jan, 6, 19, 27 Feb	1.0 oz	20.0

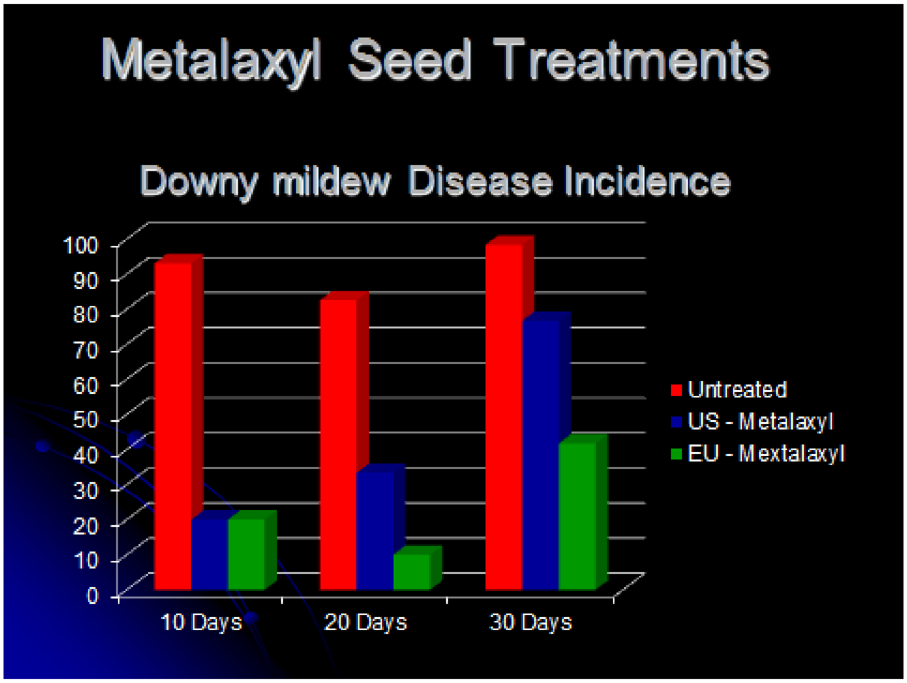
Zampro 4.38SL	31 Jan, 6, 19, 27 Feb	14.0 fl oz	20.0
Ridomil Gold 480SL	25-Jan	1.25 pt	25.0
+ Quadris 2.08SC		10.6 fl oz	
Forum	31-Jan	6.0 fl oz	
Prophyt 6.64SL	6-Feb	4.0 pt	
Prophyt 6.64SL	19-Feb	4.0 pt	
+ Presidio 4SC		4.0 fl oz	
Zampro 4.38SL	27-Feb	14.0 fl oz	
Revus 250SC	31 Jan and 19 Feb	8.0 fl oz	37.5
Ranman	31 Jan, 6, 19, 27 Feb	2.75 fl oz	37.5
LifeGard WG	31 Jan and 19 Feb	2.25 oz	40.0
Revus 250SC	6 and 27 Feb	8.0 fl oz	
Serenade ASO	25-Jan	4.0 qt	42.5
Serenade ASO	31 Jan and 19 Feb	4.0 qt	
Sonata	6 and 27 Feb	4.0 qt	
Serenade ASO	31 Jan, 6, 12, 19 Feb	4.0 qt	55.0
Serenade ASO	31 Jan and 19 Feb	4.0 qt	57.5
Sonata	6 and 27 Feb	4.0 qt	
LifeGard WG	31 Jan, 6, 19, 27 Feb	2.25 oz	60.0
Serenade ASO	25-Jan	4.0 qt	67.5
Serenade ASO	25, 31 Jan; 6, 19, 27 Feb	4.0 qt	67.5
Untreated control			82.5

LSD (Least Significant Difference, P = 0.05) = 11.8.

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.



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



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

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%.

We have established seed washing protocols to detect and quantify oospores on spinach seed from commercial seed lots. We are also expanding our efforts to examine the role of oospores on the epidemiology of the downy mildew pathogen by developing protocols for generating oospores in mixed infections in a detached leaf assay.

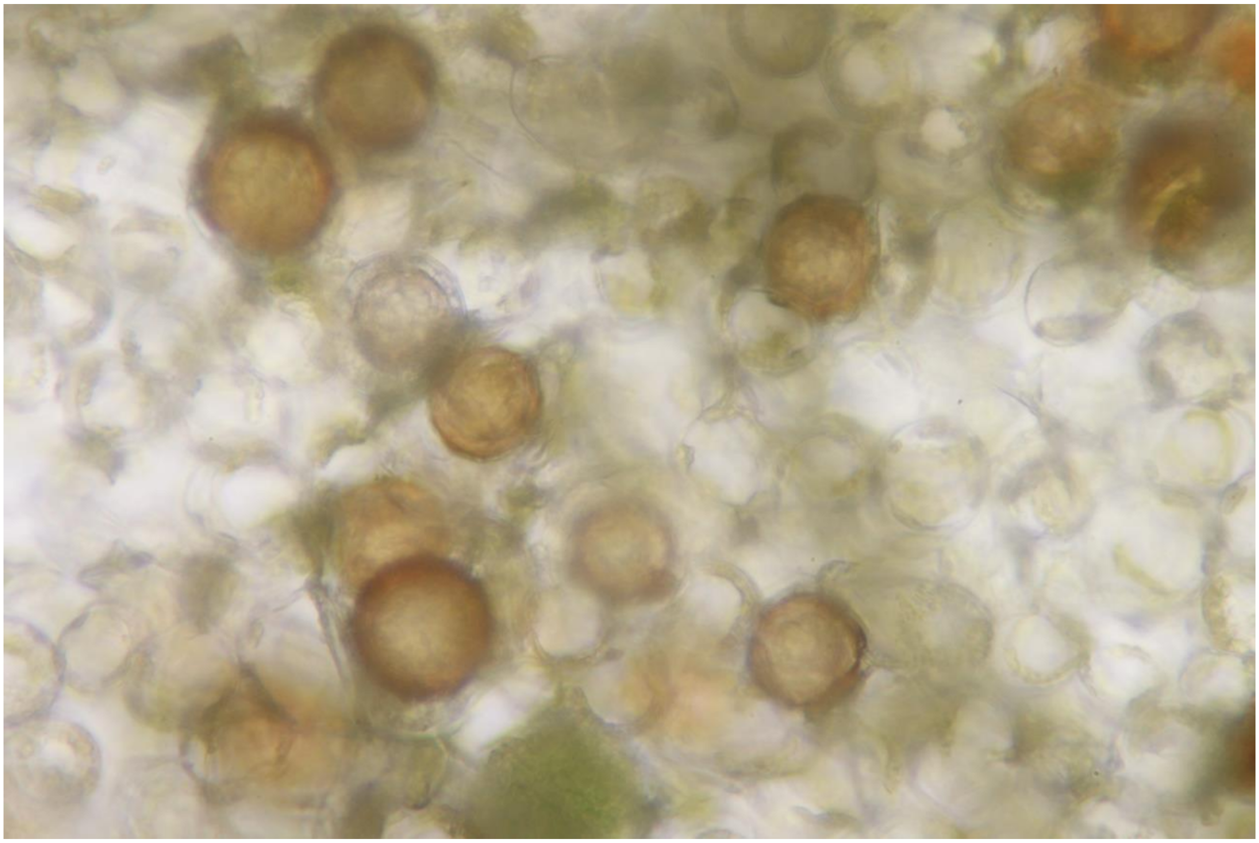
Evaluation of a soil-less detached leaf inoculation method for characterizing the downy mildew disease reaction on differential cultivars.

Cultivar	Method	Plant Part	Expected	Observed
Viroflay	WP	Cotyledons	+	+
		True leaf	+	+
	DL	Cotyledons	+	+
		True leaf	+	+
NIL5	WP	Cotyledons	+	+
		True leaf	+	+
	DL	Cotyledons	+	+
		True leaf	+	+
NIL3	WP	Cotyledons	+	+
		True leaf	+	+
	DL	Cotyledons	+	+
		True leaf	+	+
NIL4	WP	Cotyledons	+	+
		True leaf	+	+
	DL	Cotyledons	+	+
		True leaf	+	+
NIL6	WP	Cotyledons	(-)	(-)
		True leaf	(-)	(-)
	DL	Cotyledons	(-)	(-)
		True leaf	(-)	(-)
NIL1	WP	Cotyledons	-	-
		True leaf	-	-
	DL	Cotyledons	-	-
		True leaf	-	-
NIL2	WP	Cotyledons	+	+
		True leaf	+	+
	DL	Cotyledons	+	+
		True leaf	+	+
Califlay	WP	Cotyledons	+	+
		True leaf	+	+
	DL	Cotyledons	+	+
		True leaf	+	+
Whale	WP	Cotyledons	+	+
		True leaf	+	+
	DL	Cotyledons	+	+

		True leaf	+	+
Pigeon	WP	Cotyledons	-	-
		True leaf	-	-
	DL	Cotyledons	-	-
		True leaf	-	-
Caladonia	WP	Cotyledons	-	-
		True leaf	-	-
	DL	Cotyledons	-	-
		True leaf	-	-
Meerkat	WP	Cotyledons	-	-
		True leaf	-	-
	DL	Cotyledons	-	-
		True leaf	-	-
Hydrus	WP	Cotyledons	-	-
		True leaf	-	-
	DL	Cotyledons	-	-
		True leaf	-	-

Set-up to evaluate whole plant and detached-leaf inoculation approaches.

We are evaluating the role of oospores in epidemiology of the downy mildew pathogen on spinach. We have been able to observe oospores in inoculation tests with mixtures of specific isolates of the pathogen. Thus, this will allow us to evaluate a wide range of parameters on how oospores develop, how the viability can be reduced, and how important they are in the development of epidemics.



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

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: https://doi.org/10.1094/PDIS-05-17-0781-RE.

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: https://doi.org/10.1094/PDIS-06-17-0859-RE.

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. Plos One: https://doi.org/10.1371/journal.pone.0188745

Feng, C., Bluhm, B. H., Shi, A., and J. C. Correll. 2018. Molecular markers linked to three spinach downy mildew disease resistance loci. Euphytica (submitted).

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