

Project Title: Race diversity and the biology of the spinach downy mildew pathogen
CLGRB Annual Report
April 1, 2019 to March 31, 2020
June 8, 2020

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Abstract:

Spinach downy mildew disease pressure remained relatively low in the 2019 season but a large number of samples were collected in early 2020 (Jan.-Mar.) in the Yuma and Imperial Valleys. A novel virulence type, designated UA2020-01E (also referred to as the Dallas type isolate) was identified among numerous isolates collected in early 2020 from the Yuma and Imperial Valleys and Maine. The 01-E type isolate was able to infect many of the newer race 1-17 resistant varieties. However, some older and newer varieties that have the RPF3 resistance gene were resistant to this new novel type isolate. The isolate caused considerable concern among growers. Working with the International Working Group on Peronospora (IWGP) in the Netherlands, it was decided that this isolate should be examined in a ringtest by a broader group to determine if this isolate should be designated as a new race (race 18). We distributed the isolate to the various labs for consideration. This novel strain is currently being evaluated to determine if it will be nominated for a race designation. Although the 01-E type isolate was widespread in the Yuma and Imperial Valleys, it did not appear to be present in our Yuma variety trial held at the University of Arizona Field Station. Work with this deviating isolate is currently underway.

Objectives

1. With Steve Koike's departure, we have worked closely with growers, seed company personnel, and PCA's to maintain a service to identify and characterize races of the downy mildew pathogen as they appear throughout the year. This effort is exceptionally labor intensive, but believe it is critical for the industry.
2. Development and evaluation of detached leaf assay for race typing and identification. This effort could greatly expedite the race identification process and reduce costs.
3. Screen spinach germplasm from plant introduction collections (PIs), advanced breeding lines, and newer (especially race 1-17 resistant) commercially released material for resistance to various contemporary races of downy mildew.
4. Establish sentinel plots in Salinas, CA, and Yuma, AZ, to evaluate variety performance based on naturally occurring downy mildew pressure.
5. Examine organic products for their effectiveness in reducing downy mildew on spinach under greenhouse and field conditions.
6. Development and evaluation of a bioassay to assess quantitative (QTL-type) resistance.

Objectives 1 and 3

Similar to the two previous years, mildew activity was relatively low for 2019. Isolates of race 16 and 17 were identified along with a number of novel type isolates (**Table 1**). Several differentials were resistant to most of the novel isolates, with the exception of the more recently found isolate UA2020-01E. Isolates that were able to infect the differential Hydrus were examined in more detail (**Table 2**). The novel isolate UA201843D, which was collected in 2018, was examined in more detail to determine which varieties were susceptible (**Table 3**).

There was a lot of mildew activity in early 2020. A total of 20 isolates were recovered and examined between January 2020 and March 2020. Of the isolates examined, race 14 and 17 were identified. However, 12 of the isolates collected during this time period were identified as the UA2020-01E type, which was able to infect many of the race 1-17 resistant varieties (**Table 4 and 5**). The UA2020-01E type isolate was identified on a wide range of varieties from the Yuma and Imperial Valleys. Interestingly, a single isolate from Maine, collected in mid-December 2019, was also identified as the UA2020-01E novel isolate type.

A summary of the disease reactions of races 1-17 and UA2020-01E is included for comparison (**Table 6**). A wide range of commercial varieties were examined for their resistance to the novel UA2020-01E isolate (**Table 7**).

A number of isolates (**Table 8**) were examined for their DNA fingerprint to determine if they were all identical (and represented a single clone) or were genetically diverse (**Figure 1**). The

Table 1. Isolates collected in 2019.

Isolate	host	ST	Viro	NIL5	NIL3	NIL4	NIL6	NIL1	NIL2	Pigeon	Caladonia	Meerkat	Hydrus
UA201843D	El Prado	CA	+	+	+	+	+	+	+	+	+	-	(+)
UA201851B	Corvair	MA	+	+	-	+	+	+	+	+	-	(+)	-
UA201904	RZ51-359	CA	+	+	+	+	+	+	-	-	-	-	(+)
UA201908M	Shelby	AZ	+	+	+	+	+	+	+	+	+	-	-
UA201908L	RZ51-359	AZ	+	+	+	+	+	+	+	+	+	-	-
UA201908E	SilverWhale	AZ	+	-	+	+	+	+	+	+	+	-	-
UA201913	Escalade	FL	+	+	+	-	+	+	+	+	+	-	-
UA201915	Meerkat	CA	+	+	-	+	-	-	+	+	-	+	-
UA201938A	Fantail	CA	+	+	+	+	+	+	+	+	+	-	-
UA201938B	Kodiak	CA	+	+	+	+	+	+	+	+	+	-	-
UA201941A	Cocopah	CA	+	+	+	+	+	+	+	+	+	-	(+)
UA201941B	Bonobo	CA	+	+	-	+	+	+	+	+	-	+	-
UA201941C	Melville	CA	+	+	-	+	+	+	+	+	-	+	-
UA201946	Viroflay	CA	+	+	+	-	-	-	+	+	+	(+)	-
UA201951	SV3719	ME	+	+	-	+	+	+	+	+	-	+	+

-: Resistance; +: susceptible; (+): sporulation on cotyledons only

Pfs 16 (UA201915 - Binary value 357) and Pfs17 (UA201851B; 201908M; 201908L; 201938A and 2038B – Binary value - 255) were found. Other isolates are novel.

Table 2. Comparison of five isolates that can infect Hydrus.

Isolate	UA201843D		UA201904		UA201941		UA202001E		UA202006C	
	C	T	C	T	C	T	C	T	C	T
Cultivar	El Prado		RZ51-359		Cocopah		Dallas		Nevada	
Date	10/26/2018		1/23/2019		10/9/2019		1/3/2020		2/7/2020	
Origin	Salinas, CA		Bard, CA		Salinas, CA		El Centro CA		Yuma, AZ	
Tissue	C	T	C	T	C	T	C	T	C	T
Viroflay	+	+	+	+	+	+	+	+	+	+
NIL1	+	+	+	+	+	+	+	+	+	+
NIL2	+	+	-	-	+	+	+	+	+	+
NIL3	+	+	+	+	+	+	-	-	-	-
NIL4	+	+	+	+	+	+	+	+	-	-
NIL5	+	+	+	+	+	+	+	+	+	+
NIL6	+	+	+	+	+	+	+	+	+	+
Tarpy	+	+	+	+	+	+	+	+	+	+
Pigeon	+	+	-	-	+	-	+	+	-	-
Meerkat	-	-	-	-	-	-	+	+	-	-
Hydrus	(+)	-	(+)	-	(+)	-	+	+	(+)	-

-: Resistance; +: susceptible; (+): sporulation on cotyledons only. C: cotyledons, T: true leaves

Table 3. Disease reactions of spinach differentials to UA201843D

Variety	Reaction	Variety	Reaction
Flamingo	+	Sp966	(+)
PV1446	(+)	Regor	-
Antalia	(+)	Alcor	+
Hydrus	(+)	Bonobo	(+)
Shelby	+	Corvus	(+)
Virgo	(+)	Crater	-
Nevada	(+)	Formax	(+)
Wombat	(+)	Lorikeet	-
El Prado	(+)	Minkar	-
Serpens	-	Nembus	-
Volans	-	Regor	-
Caelium	-	Tabit	-
Meerkat	-	Monterey	-
Rigel	(+)	PV1444	(+)

-: Resistance; +: susceptible; (+): sporulation on cotyledons only. Tested twice.

Table 4. Spinach downy mildew isolates tested in 2020

Isolate	Cultivar	Location	Date		
			received	Phenotyping	Genotyping
UA201951	SVB 3719	Winslow, ME	12/18/2019	UA202001E	Done
UA202001A	172	El Centro, CA	12/30/2019	UA202001E	Done
UA 202001B	El Rio	El Centro, CA	12/30/2019	UA202001E	Done
UA202001E	Dallas	El Centro, CA	1/3/2020	UA202001E	Done
UA202003B	El Giga	Yuma, AZ	1/16/2020	UA202001E	Done
UA202004A	Dallas	Yuma, AZ	1/21/2020	UA202001E	Done
UA202004B	Dracus	Yuma, AZ	1/21/2020		Done
UA202004C	Nevada	Yuma, AZ	1/21/2020	UA202001E	Done
UA202004D	Cocopah	Yuma, AZ	1/23/2020	Not viable	Done
UA202004E	Nevada	Yuma, AZ	1/23/2020		Done
UA202006A	El Giga	Yuma, AZ	2/6/2020	UA202001E	
UA202006B	El Giga	Yuma, AZ	2/6/2020	UA202001E	
UA202006C	Nevada	Yuma, AZ	2/6/2020	UA202006C	
UA202007A	51-367	Yuma, AZ	2/13/2020	UA202001E	
UA202007C	El Giga	Yuma, AZ	2/13/2020	UA202001E	
UA202008A	Cocopah	Yuma, AZ	2/20/2020	UA202001E	
UA202008D	Trial Boss	Yuma, AZ	2/22/2020	Pfs17	
UA202009E	Trailboss	Yuma, AZ	2/27/2020	Pfs14	
UA202009F	Viroflay	Yuma, AZ	2/27/2020	Pfs17	
UA202010B	Bandera	Yuma, AZ	3/5/2020	UA202006C	

Table 5. List of UA202001E type isolates (as of 3/25/2020)

Isolate	Date	Origin	Cultivar
UA202001E	1/3/2020	CA	Dallas
UA201951	12/18/2019	ME	SV3719
UA202001A	12/30/2019	CA	172
UA202001B	12/30/2019	CA	El Rio
UA202003B	1/16/2020	AZ	El Gigo
UA202004A	1/21/2020	AZ	Dallas
UA202004C	1/23/2020	AZ	Nevada
UA202006	2/6/2020	AZ	El Giga
UA202006B	2/7/2020	CA	El Giga
UA202007A	2/13/2020	AZ	51-367
UA202007C	2/13/2020	AZ	El Giga
UA202008A	2/20/2020	AZ	Cocopah

Table 6. Disease reactions of spinach differential cultivars to races of *Peronospora effusa*

Pfs	Viroflay	NIL1	NIL2	NIL3	NIL4	NIL5	NIL6	Pigeon	Meerkat	Hydrus
1	+	-	-	-	-	-	-	-	-	-
2	+	-	-	+	-	-	-	-	-	-
3	+	-	-	-	-	+	-	-	-	-
4	+	-	-	+	-	+	-	-	-	-
5	+	-	-	-	+	+	-	-	-	-
6	+	-	-	+	+	+	+	-	-	-
7	+	-	-	+	+	+	-	-	-	-
8	+	+	-	-	+	+	+	-	-	-
9	+	-	-	-	+	+	+	-	-	-
10	+	+	-	+	+	+	+	-	-	-
11	+	-	+	-	+	+	-	-	-	-
12	+	+	+	-	+	+	+	-	-	-
13	+	-	+	+	+	+	+	-	-	-
14	+	+	+	-	+	+	+	+	-	-
15	+	-	-	+	-	+	-	-	-	-
16	+	-	+	-	+	+	-	+	+	-
17	+	+	+	+	+	+	+	+	(+)	-
01E*	+	+	+	-	+	+	+	+	+	+

*UA2020-01E Dallas type isolate

Figure 1. Genotyping of *Peronospora effusa* by lesions of composite isolates

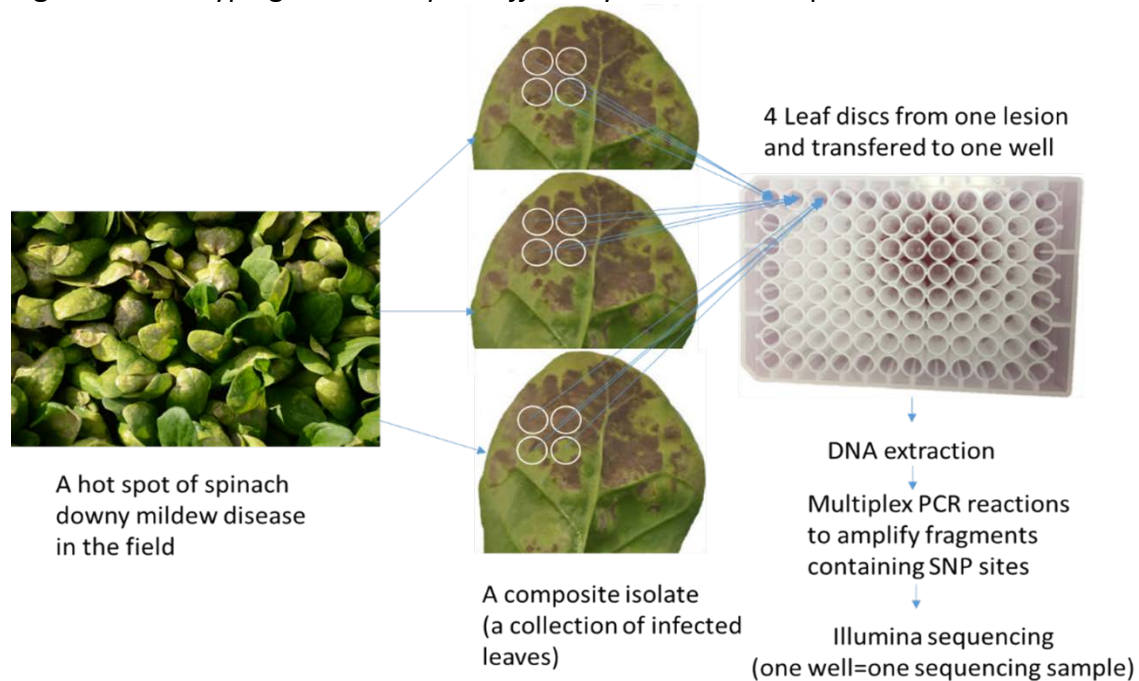


Table 7. Disease reactions of spinach varieties to UA2020-01E

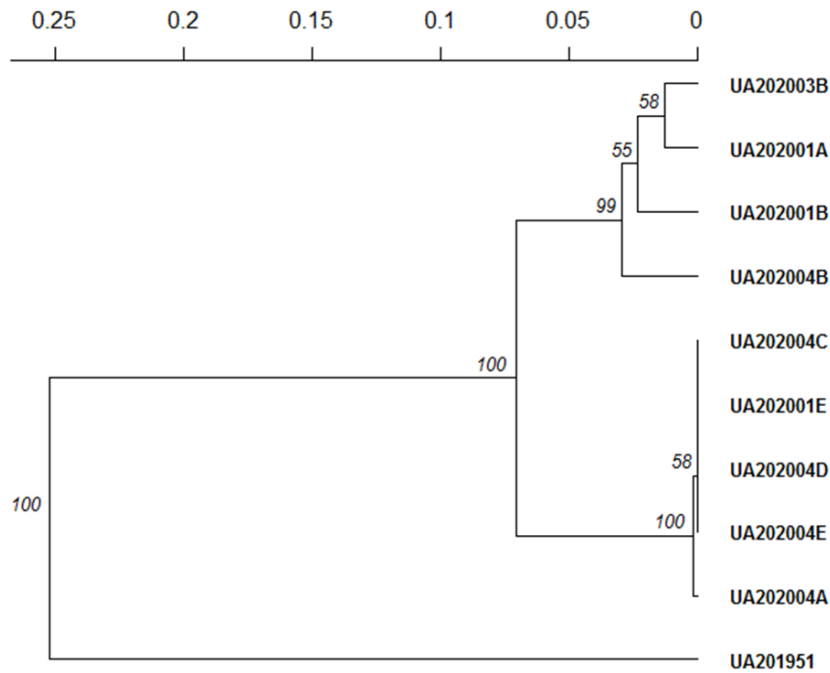
Variety	Reaction	R gene	Variety	Reaction	R gene	Variety	Reaction	R gene
Arlington	+		PV1488	+		Whale	-	RPF 3
Bandera	+		PV1512	+		Woodpecker	+	
Dallas	+		PV1514	+		Red Kitten	+	
Nevada	+		PV1515	+		51-348	+	
Dracus	-		Alcor	+		51-351	-	RPF 3
Regor	-		Crater	-		Banjo	-	RPF 3
Sculptur	+		Formax	+		Caladonia	-	RPF 3
Tabit	-		PPV1053	-		Califlay	-	RPF 3
Monterey	-		PV1206	-		Camaro	-	RPF 3
PV1444	+		PV7136	+/-		Corvair	+	
PV1448	+		SP966	+		Golden Eye	-	RPF 3
PV1446	+		Sp967	+		Lion	-	RPF 3
PV1452	+		Tasman	-	RPF 3	Monza	+	
PV1477	+		Violin	-	RPF 3	Kiowa	+	
Virgo	+		PV1506	+		Midway	+	
Minkar	-		Crosstrek	-		Bylot	+	
Supltur	+		Sp997	+		Tacoma	-	
Riverside	-		Sp999	-		Sunangel	-	
C7-613	-		El Lucio	-		Cabazon	-	
Patton	+		Magnetic	+		2636	+	
Trailboss	-		5998	+				

Table 8. Number of genotypes identified among each isolate.

Isolate	Lesion	Genotype
UA201951	15	2
UA202001A	11	9
UA202001B	4	4
UA202001E	45	1
UA202003B	12	9
UA202004A	9	2
UA202004B	6	2
UA202004C	10	1
UA202004D	3	1
UA202004E	6	1
Total	121	28

isolates were genetically diverse, with the UA2020-01E type isolate from Maine being the most different. **(Figure 2).**

Figure 2. The genetic relation among isolates of Pfs that have the UA2020-01E virulence type indicating that there is diversity among the isolates and that they are not all clonal related.



Objective 2

A detached leaf bioassay is still being examined as a method to expedite the identification of races of the downy mildew pathogen. This assay has been validated for race 13 and is currently being evaluated with race 5 and the novel isolate UA2020-01E. The disease reactions on detached leaves were identical to the disease reactions on the whole plants; thus the bioassay was validated **(Table 9).**

Objective 4

Two large-scale variety trials were conducted in September-October, 2019 in San Juan Bautista and January-February 2020. An industry Spinach Field Day was held for stakeholders on October 9, 2019, and February 26, 2020. The Spinach Field Days were well attended with over 100 people signing up for the field day. The disease reactions for the October 2019 and February 2020 are listed below.

Evaluation of spinach varieties for downy mildew resistance, San Juan Bautista, CA 2019.

Downy mildew is the most economically important disease of spinach. Approximately 50% of the total spinach produced in California and Arizona is grown organically. Thus, resistance remains a critical tool for downy mildew management in spinach production. This study was conducted at the Seminis Vegetable Seeds Research Station in San Juan Bautista, CA, in Sept-Oct 2019. A total of 70 spinach cultivars were evaluated for disease reactions to downy mildew. The plots were sprinkler-irrigated to germinate seed on 13 Sept. on beds with 84 in. between bed-centers, with each bed containing 16 lines of plants, at a seeding rate of 3.5 million seed/acre. The total plot area was 75 x 225 ft. Treatments were replicated three times in a randomized complete block design. Each replicate plot consisted of a 15 ft length of the bed. Maximum and minimum ranges (°F) of air temperature were as follows: 66.9-101.9, 34.3-55.3 from 9 Sept. to 17 Oct. There was only a trace of rain (0.01 inches) during the trial. Plants were watered with overhead sprinklers 2-3 times per week for the duration of the trial. Downy mildew was first observed in plots on 7 Oct 2019. Incidence was measured by visually estimating the disease in each of the three replicate plots per cultivar. Mean disease incidence values were calculated using the three replications.

The final evaluation for downy mildew incidence was made on 11 Oct 2019. The overall disease pressure in 2019 was very low. The low disease pressure could have been due to the age of the plants when rated (28 days from wet date), weather conditions unfavorable for disease development, and the predominant use of cultivars with resistance to races 1-17 of *Pfs*. The data in the table indicate a narrow range in disease incidence (0.0 to 8.3%) for the cultivars evaluated. In addition, there was a high level of variation between the disease ratings between replications. As a result, no significant difference was calculated in the reaction of the cultivars. This was a baby-leaf spinach planting, where tolerance for leaves infected with downy mildew would be extremely low (typically less than 3%) in a commercial planting.

Cultivar	Disease incidence ^y	Cultivar	Disease incidence ^y	Cultivar	Disease incidence ^y
SV2146VB	8.3 a ^z	C7-613	0.0 a	Patton	0.0 a
SV3580VC	8.3 a	Cabazon	0.0 a	Platypus	0.0 a
Bonobo	6.7 a	Canary	0.0 a	PV1449/Cocopah	0.0 a
Riverside	6.7 a	Colusa	0.0 a	PV1512/Bandera	0.0 a
SV2157VB	5.3 a	Corvus	0.0 a	PV1513/Dallas	0.0 a
Shelby (treated)	5.0 a	Crater	0.0 a	PV1514/Laredo	0.0 a
Yukon	5.0 a	Dracus	0.0 a	PV1515/Houston	0.0 a
Nembus	5.0 a	El Caballo	0.0 a	PV1517/Arlington	0.0 a
Acadia	3.3 a	El Lucio	0.0 a	PV1526	0.0 a
SV1864VC	3.3 a	El Prado	0.0 a	Reflect	0.0 a
Dromedary	1.7 a	El Rio	0.0 a	Regor	0.0 a
Melville	1.7 a	Escalade (treated)	0.0 a	Salamander	0.0 a
Pawnee	1.7 a	Finwhale	0.0 a	Seaside	0.0 a

Responder	1.7 a	Formax	0.0 a	SP997	0.0 a
SP999	1.0 a	Hydrus	0.0 a	Spoonbill	0.0 a
Silverwhale	0.7 a	Java	0.0 a	Sunangel	0.0 a
Alcor	0.3 a	Kiowa	0.0 a	SV6203VB	0.0 a
Fantail	0.3 a	Kodiak	0.0 a	Tabit	0.0 a
Lakeside	0.3 a	Magnetic	0.0 a	Tundra (treated)	0.0 a
Midway	0.3 a	Minkar	0.0 a	Virgo	0.0 a
Sculptur	0.3 a	Nevada	0.0 a	Viroflay	0.0 a
Antigua	0.0 a	Nun07542	0.0 a	Volans	0.0 a
Bandicoot	0.0 a	Oceanside	0.0 a		
C2-606	0.0 a	Octans	0.0 a		

^y Disease incidence (DI) was estimated based on visually inspecting a 1 x 1 m square area in the center of each plot.

^z 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 ($P = 0.05$).

Evaluation of spinach cultivars for downy mildew resistance in Yuma, AZ 2020.

A total of 70 spinach cultivars were evaluated at the University of Arizona Experiment Station in Yuma, AZ between 15 Jan - 5 Mar 2020. Seed were sown on 15-16 Jan in 80 in. beds which were formed by merging two 40 in. beds. The wet date was 17 Jan. Each bed contained 16 lines of plants, at a seeding rate of 3.5 million seed/A. Treatments were replicated three times with a randomized complete block design. Each replication consisted of 70 plots of 80 in. width by 10 ft length. The trial was flanked by single beds of the cultivars Hydrus and an SV line. During the trial period, maximum air temperature ranged from 60-84 °F and minimum from 34-58 °F, cumulative rainfall was 1.19 in. and the maximum wind peak was 30 mph. Plants were watered with overhead sprinklers 2-3 times per week for the duration of the trial.

All downy mildew disease pressure was from naturally occurring inoculum and was first observed in the plots on 24 Feb. The final evaluation for disease incidence (DI) was made on 5 Mar (48 days after planting) and is reported in the data table below. The range in mean DI for the cultivars evaluated was 0.0 to 61.7%. This was a baby-leaf spinach planting, where tolerance for leaves infected with downy mildew would be extremely low (typically less than 3%) in a commercial spinach production. *P. effusa* race 14 and 17 were identified in the trial. Although isolate UA202001E was widespread in commercial spinach production fields in the area, there was no evidence it was present in the trial.

Cultivar	Mean disease incidence (DI) ²	Cultivar	Mean DI	Cultivar	Mean DI
SV2157VB	61.7 a ^y	Virgo	0.0 j	El Lucio	0.0 j
SV1864VC	56.7 ab	Volans	0.0 j	El Prado	0.0 j
Yukon	55.0 ab	Alcor	0.0 j	El Rio	0.0 j
Red Kitten	48.3 abc	Regor	0.0 j	Magnetic	0.0 j
Seaside	48.3 abc	Minkar	0.0 j	5998	0.0 j
C7-613	40.0 bcd	Nembus	0.0 j	Java	0.0 j
SV2146VB	40.0 cd	Tabit	0.0 j	Midway	0.0 j
Lakeside	38.3 cd	Corvus	0.0 j	Melville	0.0 j
Escalade ^x	37.5 cde	Crater	0.0 j	Bylot	0.0 j
Shelby ^x	36.7 cde	Dracus	0.0 j	Tacoma	0.0 j
Reflect	35.0 cde	Formax	0.0 j	Kodiak	0.0 j
Acadia	31.7 def	Sculptur	0.0 j	Sunangel	0.0 j
Oceanside	30.0 defg	Patton	0.0 j	Fantail	0.0 j
Riverside	28.3 defg	Nevada	0.0 j	Bonobo	0.0 j
SV3580VC	26.7 defg	PV1514/Laredo	0.0 j	Cabazon	0.0 j
Tundra ^x	21.7 efg	Colusa	0.0 j	Spoonbill	0.0 j
Califlay	17.5 fgh	PV1513/Dallas	0.0 j	Bandicoot	0.0 j
C2-606	15.0 ghi	PV1512/Bandera	0.0 j	Finwhale	0.0 j
Responder	15.0 ghi	PV1515/Houston	0.0 j	Pawnee	0.0 j
Silverwhale	5.0 hij	PV1517/Arlington	0.0 j	PV1506	0.0 j
Viroflay	3.3 hij	PV1449/Cocopah	0.0 j	2636	0.0 j
Trailboss	1.0 hj	Crosstrek	0.0 j	Platypus	0.0 j
PV1506	1.0 hj	SP997	0.0 j		
Whale	0.7 hj	SP999	0.0 j		

²Downy mildew incidence was evaluated by visually estimating the percent of plants infected in each of the three replicate plots per cultivar. Mean DI was calculated by averaging the three replicates.

^y Analysis of variance (ANOVA) was performed ($P \leq 0.05$) followed by Post Hoc analysis using Fisher's least significant difference (LSD) test. Mean DI scores with the same letter are not significantly different as determined by Fisher's LSD test ($P \leq 0.05$).

^x Seed were treated with a standard thiram/metalaxyl seed treatment.

Objective 5

We continue to examine a wide range of organic biofungicides for efficacy for downy mildew. Over 20 materials were examined with little to no efficacy relative to conventional fungicides. We have received several newer materials recently, and these materials are being evaluated.

Fungicides evaluated for downy mildew control in the field, greenhouse, or both.

Material
Biofungicide
Actinovate
Aliette
Biospecific
Cueva
Double Nickle
Duolif
GC-Pro1
GC-Pro-2
GWN-10580
Gowan Experimental
Iso NPK
Nordex
Oxidate
Oxiphos
Procidic
Procidic 2
Purgrow
Sea Shield
Serenade
Serenade Max
T22HC
Trifecta
Vesta/Blue Cal
Sea Shield
Timorex

Conventional
Aliette
Blockage
Mildecut L
Mildecut H
Orondis
Ranman
Ranman STK73
Ridomil Gold
Revus
Zampro

Objective 6. Efforts are underway to evaluate quantitative resistance to the downy mildew pathogen. Such an approach involves a much higher level of precision to evaluate this type of resistance, and various variables, including inoculum level, temperature, plant nutrition, etc., are being evaluated.

Table 9. Disease responses of spinach differential cultivars to the spinach downy mildew pathogen [*Peronospora effusa* race 13 (*Pfs* 13)] maintained on whole plants (WP) or detached leaves (DL) in a whole plant or detached leaf bioassay. Disease response of the differential cultivars inoculated using *Pfs* maintained exclusively on the detached leaves are also presented.

Cultivars	DM response following DL assay ^x					
	<u>Expected DM response^w</u>	<u><i>Pfs</i> maintained on WP^y</u>			<u><i>Pfs</i> maintained on DL^z</u>	
		WP	DL	WP	DL	WP
Virolfay	+	+	+	+	+	
NIL2	+	+	+	+	+	
NIL3	+	+	+	+	+	
NIL4	+	+	+	+	+	
NIL5	+	+	+	+	+	
NIL6	+	+	+	+	+	
Whale	+	+	+	+	+	
Califlay	+	+	+	+	+	
NIL1	-	-	-	-	-	
Caladonia	-	-	-	-	-	
Pigeon	-	-	-	-	-	
Hydrus	-	-	-	-	-	
Meerkat	-	-	-	-	-	

^w Known downy mildew response of the spinach differential cultivars as reported in Feng et al., 2014, 2018 following the standard whole plant inoculation method. A plant showing chlorosis and sporulation on cotyledons, true leaves, or both was classified as susceptible (+). A plant without chlorosis or sporulation was rated as resistant (-). A spinach cultivar was classified as susceptible if more than 85% of the plants were diseased (+) or classified as resistant if less than 15% of plants were diseased (-).

^x Downy mildew disease response of each cultivar following the whole plant (WP) and detached leaf (DL) inoculation assay conducted to validate the detached leaf inoculation assay.

^y *Pfs* inoculum was prepared weekly on the whole plants of the susceptible cultivar Virolfay in a separate tray. The disease response report is from the three independent inoculation tests for each cultivar

^z *Pfs* infected detached leaves on the Petri dishes were only used to prepare spore suspension and were used to inoculate the detached leaf of differential cultivars. The disease response report is from the two independent inoculation tests for each cultivar.

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