

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

Spinach downy mildew disease pressure remained relatively low in the 2020 season but a large number of samples were collected in early 2020 (Jan.-Mar.) in the Yuma and Imperial Valleys. A total of 42 isolates have been characterized. Isolate UA202047 (Originated from Spain) could infect NIL2, 3, 4, 5, Pigeon, Caladonia, Meerkat, but not NIL1, 6, and Hydrus. The disease reactions of differentials to this isolate were identical to that of differentials to seven isolates found in the US during 2015 to 2018. This type of isolates was denominated as race Pfs 18. Isolate UA202001E could infect NIL1, 2, 4, 5, 6, Pigeon, Meerkat, and Hydrus, but not NIL3, Whale, and Caladonia. This type of isolates has been denominated as Pfs 19 by the International Working Group on Peronospora (IWGP) in April 2021. Five novel strains were also identified, four of which could infect the cotyledons of Hydrus, a cultivar with resistance to Pfs 1-18. Sixty-eight spinach cultivars were tested in controlled environment with the two new races, Pfs 18 and Pfs 19, and five novel strains. These cultivars were also evaluated in field condition for resistance to downy mildew disease in Yuma, AZ.

Objectives

1. 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. Evaluate advanced breeding lines, and newer commercially released material for resistance to new races and novel strains of downy mildew.
3. Establish sentinel plots in Yuma, AZ, to evaluate variety performance based on naturally occurring downy mildew pressure.

Results

Objectives 1 and 2

Since the end of 2019, we have collected 87 isolates, 47 isolates have been tested (Table 1), but five isolates were not viable. Out the 42 isolates, two are under testing. Three isolates were mixture that did not cause clear disease reaction patterns on differentials. Three isolates each were identified as Pfs 14 and Pfs 17, one isolate each was identified as Pfs 4 and Pfs 7. Other isolates were identified as new races or novel strains with unique virulence phenotypes on the differentials.

Two new races were identified, which showed unique virulence phenotypes on the differentials that were different from all named 17 races of *P. effusa* (Table 2). Isolate UA202047 (Originated from Spain) could infect Viroflay, NIL2, 3, 4, 5, Pigeon, Caladonia, Meerkat, but not NIL1, 6, and Hydrus. The disease reactions of differentials to this isolate were identical to that of differentials to seven isolates found in the US during 2015 to 2018, including UA201502, UA201526A, UA201702A, UA201703D, UA201707C, UA201709G, UA201818B. This type of isolates has been denominated as Pfs 18 by the International Working Group on Peronospora (IWGP) in Apr. 2021. Pfs18 causes severe damage in the E.U. but has not been found in the US after 2018. Isolate UA202001E could infect Viroflay, NIL1, 2, 4, 5, 6, Pigeon, Meerkat, and Hydrus, but not NIL3, Whale, and Caladonia. Eighteen isolates collected from Arizona (17 from Yuma, and one from Phoenix), and one isolate from Maine, cause identical disease reactions on the differentials. This type of isolates has been denominated as Pfs 19 by the IWGP in Apr. 2021. Since Pfs 19 could overcome the widely used resistance gene of Hydrus that provides resistance to Pfs 17, Pfs 19 type isolates could potentially cause severe damage in spinach production. The resistance locus RPF3 (and maybe a few uncharacterized resistant loci) can provide resistance to Pfs 19. Within each isolate of Pfs 19, there could be no genetic variation (only one genotype of multiple lesions of that isolate, e.g. UA202001E, UA202004C, UA202004D, and UA202004E) or abundant genetic variation (multiple genotypes within an isolate, e.g. UA202001A and UA202004A). And a few isolates of Pfs 19 had minor phenotype variation. For example, the disease reactions of differentials to the isolate UA202105C (collected from spinach cultivar Tabit from Yuma, AZ) were identical to that of differentials to UA202001E, thus UA202105C was identified as Pfs 19 type. However, UA202105C could infect Tabit, which was resistant to UA202001E.

There were five novel strains identified in 2020 (Table 3), which had virulence phenotypes different from all known races or unique strains previously identified. Isolate UA202025A could infect Viroflay, NIL2, 4, 6, Pigeon, and cotyledons of Meerkat, but not NIL1, 3, 5, and Hydrus. Other four isolates could infect the cotyledons but not the true leaves of cultivar Hydrus, which is resistant to all known 17 races of *Peronospora effusa*. Isolate UA202006C could infect Viroflay, NIL1, 2, 5, 6, the cotyledons of Hydrus, but not NIL3, 4, Pigeon, Caladonia, Meerkat. Isolate UA202043A could infect NIL1, 2, 6, Pigeon, the cotyledons of Meerkat and Hydrus, but NIL3, 4, 5, and Caladonia (containing RPF3) were resistant. Isolate UA202044A could infect all the differentials, but Meerkat and Hydrus only had cotyledons infection. Isolate UA202044B could

infect NIL1, 2, 3, 4, 6, Pigeon, Caladonia, and the cotyledons of Meerkat and Hydrus, only NIL5 was resistant.

Table 1. Isolates tested from January 2020 to May 2021.

Isolate	Host Cultivar	Origin	Date Received	Race ID
UA201951	SVB3719	Winslow, ME	12/18/2019	Pfs 19
UA202001A	172	El Centro, CA	12/30/2019	Pfs 19
UA202001B	El Rio	El Centro, CA	12/30/2019	Pfs 19
UA202001E	Dallas	El Centro, CA	1/3/2020	Pfs 19
UA202003B	El Giga 997	Yuma, AZ	1/16/2020	Pfs 19
UA202004A	Dallas	Yuma, AZ	1/21/2020	Pfs 19
UA202004C	Nevada	Yuma, AZ	1/21/2020	Pfs 19
UA202004D	Cocopah	Yuma, AZ	1/23/2020	not viable
UA202006	El Giga	Yuma, AZ	2/6/2020	Pfs 19
UA202006B	El Giga	Imperial Valley, CA	2/7/2020	Pfs 19
UA202006C	Nevada	Yuma, AZ	2/7/2020	novel
UA202007A	51-367	Yuma, AZ	2/13/2020	Pfs 19
UA202007C	El Giga	Yuma, AZ	2/13/2020	Pfs 19
UA202008A	Cocopah	Yuma, AZ	2/20/2020	Pfs 19
UA202008D	Trailboss	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
	Space/kookaburrr			
UA202025A	a	Falmouth, ME	6/19/2020	UA202025A
UA202027	Nun07542	Salinas, CA	7/1/2020	Pfs14
UA202028	Viroflay	Salinas, CA	7/7/2020	mixture
UA202041B	Kookaburra	Burlington, VT	10/9/2020	not viable
UA202041C	Plymouth	Le Roy, NY	10/9/2020	not viable
UA202041D	SV1714 (SV1)	Le Roy, NY	10/9/2020	not viable
UA202043A	Corvair	Union Bridge, MD	10/21/2020	UA202043A
UA202044A	Auroch	Amherst, MA	10/26/2020	UA202044A
UA202044B	Kookaburra	Gilford, NH	10/28/2020	UA202044B
UA202045	Corvair	Amherst, MA	11/4/2020	Pfs14
UA202046A	Crater	King city, CA	11/13/2020	UA202044B
UA202047		Netherlands	11/18/2020	Pfs 18
UA202048	Kookaburra	Ipswich, MA	11/25/2020	UA202044A
UA202051	Lacerta	Yuma, AZ	12/15/2020	Pfs 19
UA202052	Crater	Yuma, AZ	12/30/2020	UA202044B
UA202004	Crater	Yuma, AZ	1/25/2021	Pfs 19
UA202105B	Nevada	Yuma, AZ	2/2/2021	Pfs 19
UA202105C	Tabit	Yuma, AZ	2/2/2021	Pfs 19
UA202107A	3592	Yuma, AZ	2/24/2021	UA202044A
UA202111A	Trailboss	Yuma, AZ	3/15/2021	Pfs 7
UA202111C	Minkar	Yuma, AZ	3/18/2021	Pfs 4
UA202114A	Lacerta	Yuma, AZ	3/29/2021	Pfs 19

UA202114B	Lacerta	Yuma, AZ	3/31/2021	Pfs 19
UA202117A	Spiro	Salinas, CA	4/20/2021	mixture
UA202117B	Gazelle	Sackets Harbor, NY	4/23/2021	UA202044A
UA202119	Racoon	Santa Barbara	5/5/2021	not viable
UA202119B	Aries	Salinas, CA	5/7/2021	testing
UA202120	Tundra+Sioux	North Easton, MA	5/14/2021	testing

Table 2. Disease reactions of differentials to the 19 races of *Peronospora effusa*

Pfs race	Viroflay	NIL5	NIL3	NIL4	NIL6	NIL1	NIL2	Pigeon	Caladonia	Meerkat	Hydrus
1	+	-	-	-	-	-	-	-	-	-	-
2	+	-	+	-	+	-	-	-	-	-	-
3	+	+	-	-	-	-	-	-	-	-	-
4	+	+	+	-	-	-	-	-	-	-	-
5	+	+	-	+	-	-	-	-	-	-	-
6	+	+	+	+	+	-	-	-	-	-	-
7	+	+	+	+	-	-	-	-	-	-	-
8	+	+	-	+	+	+	-	-	-	-	-
9	+	+	-	+	+	-	-	-	-	-	-
10	+	+	+	+	+	+	-	-	-	-	-
11	+	+	-	+	-	-	+	-	-	-	-
12	+	+	-	+	+	+	+	-	-	-	-
13	+	+	+	+	+	-	+	-	-	-	-
14	+	+	-	+	+	+	+	+	-	-	-
15	+	+	+	-	-	-	-	-	+	-	-
16	+	+	-	+	-	-	+	+	-	+	-
17	+	+	+	+	+	+	+	+	+	(+)	-
18	+	+	+	+	-	-	+	+	+	+	-
19	+	+	-	+	+	+	+	+	-	+	+

Table 3. Disease reactions of differentials to five novel strains of *Peronospora effusa*

Isolate	Viroflay	NIL5	NIL3	NIL4	NIL6	NIL1	NIL2	Pigeon	Caladonia	Meerkat	Hydrus
UA202006C	+	+	-	-	+	+	+	-	-	-	(+)
UA202025A	+	-	-	+	+	-	+	+	-	(+)	-
UA202043A	+	-	-	-	+	+	+	+	-	(+)	(+)
UA202044A	+	+	+	+	+	+	+	+	+	(+)	(+)
UA202044B	+	-	+	+	+	+	+	+	+	(+)	(+)

Planting resistant cultivars is the most economical way to manage spinach downy mildew disease, especially for organic spinach production. However, the fast emergence of new races or novel strains may breakdown the resistance deployed. It is critical to find out the disease reactions of newly released cultivars to the new races and novel strains. A total of 68 cultivars, most have

been tested in the 2020 field trial in Yuma, AZ were tested with the two new races Pfs18 and Pfs 19, and five novel strains in controlled environment. Each isolate has been examined in two independent tests (except isolate UA202105C was tested only once). The results of the 68 cultivars to these two races and five novel strains were listed in table 4. The diseases reactions of differentials to isolate UA202001E and UA202105C are identical, but can be differentiated by cultivar Tabit and a few other cultivars (Table 4).

Table 4. Disease reactions of 70 lines to two races and five novel strains of *Peronospora effusa*

Genotype	Pfs 18	Pfs 19	UA202043A	UA202044A	UA202046	UA202048	UA202105C
Formax	+	+	-	-	-	(+)	(+)
Califlay	+	-	-	+	+/-	+	-
Sculptur	-	+	(+)/-	-	-	(+)	(+)
Red Kitten	+	+	+	+	+	+	+
Whale	+	-	-	+	-	+	-
Riverside	+	-	-	+	+	+	-
Lakeside	+	-	-	-	+	+	-
Seaside	+	-	-	+	+	+	-
Oceanside	-	-	-	-	+	+	-
C2-606	-	-	-	+	+	+	-
Houston	-	+	(+)	-	-	(+)/-	(+)
Arlington	-	+	(+)	-	+/-	(+)/-	(+)
Cocopah	-	+	(+)	-	+/-	(+)	(+)
PV1506	-	+	(+)	-	+/-	(+)	(+)
Viroflay	+	+	+	+	+	+	+
SP997	-	+	(+)	-	-	(+)	+
SP999	-	-	-	-	-	-	-
El Lucio	-	-	-	-	-	-	-
El Prado	-	+	(+)	-	(+)	(+)	+
El Rio	-	+	(+)	-	(+)	(+)	+
Magnetic	+	+	(+)	+	(+)	(+)	+
5998	+	+	(+)	+	+/-	(+)	+
Java	+	+	(+)	+	(+)	(+)	+
Midway	+	+	(+)	+	(+)	(+)	+
Melville	+	+	+	+	(+)	(+)	+
SV2146VB	-	+	+	+	+	+	+
SV2157VB	-	+	+	+	+	+	+
SV3580VB	-	+	+	+	+	+	+
SV1864VB	+	-	-	+	+	+	-
Bylot	-	+	(+)	+	(+)	(+)	+
Tacoma	+	-	-	-	-	(+)	-
Kodiak	-	+	+	-	(+)	(+)	+
Sunangel	-	-	-	+/-	+/-	+/-	+
Fantail	+	+	-	+	(+)	(+)	+
Silverwhale	-	-	-	+	(+)	+	-
Bonobo	-	+	(+)	+	(+)	(+)	+

Cabezon	(+)	-	-	+	(+)	(+)/-	-
Spoonbill	(+)	-	-	+	(+)	(+)	-
Bandicoot	+/-	+	-	+	(+)	(+)	+
Finwhale	(+)	+	-	+	-	(+)	+
Virgo	-	+	-	-	+/-	(+)	(+)
Volans	-	+	-	-	(+)	(+)	(+)
Alcor	-	+	(+)	-	+/-	(+)	+
Regor	+	-	-	-	-	-	-
Minkar	-	-	-	-	-	-	-
Nembus	-	-	-	-	-	-	-
Tabit	-	-	+	-	-	-	+
Corvus	-	+	(+)	-	+/-	(+)	(+)
Crater	-	-	-	-	+	(+)	-
Dracus	+/-	-	-	-	-	(+)	-
Viroflay	+	+	+	+	+	+	+
Pawnee	+	-	-	-	-	-	-
Tundra	-	+	+	+	+	+	(+)
Escalade	+	-	-	+	+	+	-
Acadia	-	+	+	+	+	+	+
Shelby	-	+	+	+	+	+	+
Yukon	+	-	+	-	+	+	-
PV1506	-	+	(+)	-	-	(+)	(+)
2636	+	+	(+)	-	+/-	(+)	+
Platybus	+	+	(+)	+	+/-	(+)	+
C7-613	+	-	-	+	+	+	+/-
Patton	+	+	(+)	+	+	(+)	+
Reflect	-	+	+	+	+	+	+
Responder	+	-	-	+	+	+	-
Trailboss	+	-	-	-	-	(+)	-
Nevada	-	+	(+)	-	+/-	(+)	(+)
Laredo	-	+	(+)	-	(+)	(+)	(+)
Colusa	-	+	(+)	-	+/-	(+)	(+)
Dallas	-	+	(+)	-	+/-	(+)	(+)
Bandera	-	+	(+)	-	+/-	(+)	+

Objective 3. Evaluation of spinach cultivars for downy mildew resistance in Yuma, AZ 2021.

Downy mildew (*Peronospora effusa*) is an economically important disease of spinach (*Spinacia oleracea*). Increased demand and production of fresh spinach continues to coincide with disease pressure. Low levels of disease incidence can lead to unmarketable spinach, creating strain on the industry. Downy mildew is effectively controlled with fungicides and resistant cultivars in conventional fields, but deployment of resistant spinach cultivars is the only means of control available for organic spinach production, which represents about half of the U.S. production. Currently, there are 19 named races of *P. effusa*, however isolates with variations in resistance are continuously identified and the biology behind the emergence of new races is not clearly understood. This study was conducted to assess *P. effusa* resistance in different commercial spinach cultivars. A total of 70 spinach cultivars were evaluated at the University of Arizona, Yuma Center of Excellence for Desert Agricultural (YCEDA) between 7 Jan - 25 Feb 2021. Seed was sown 7-8 Jan and wet on 9 Jan. Each plot was 15 ft by 6 ft and seed was planted at a density of 4 million seed/A. Beds were approximately 600 ft in length and broken into three treatments with 10 rows of 7 plots across. Each treatment consisted of 70 plots with a randomized block design. The trial was flanked by single beds of the cultivar Woodpecker and a mixture of cultivars. Maximum air temperature ranged from 57-85 °F and minimum from 36-55 °F, cumulative rainfall was 1.31 in., and the maximum wind peak was 33 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 on 8 Feb. Disease incidence (DI) was evaluated on 24-25 Feb when plants were 46-47 days old. Downy mildew disease pressure was high as indicated by the DI observed on several cultivars and the range of DI was from 0 to 100%. Several cultivars with disease symptoms were inspected for the presence of *P. effusa* oospores. Oospores were observed in 12 of the 18 cultivars observed indicating that both *P. effusa* mating types were present in this location during the trial.

Cultivar	Mean disease incidence (DI)	Cultivar	Mean DI	Cultivar	Mean DI
606	100.0 a	Parakeet	11.7 jkl	Houston	0.3 l
Red Kitten	98.3 ab	Trialboss	11.0 jkl	Laredo	0.3 l
SV2157VB	95.0 bc	1038	10.0 jkl	Parentie	0.3 l
Lakeside	93.3 c	Midway	10.0 jkl	Alameda	0.3 l
SV2146VB	83.3 d	999	8.7 jkl	1059	0.0 l
DS40001	78.3 de	SV5998	8.7 jkl	Colusa	0.0 l
Responder	76.7 de	EXP 3548	6.7 jkl	Baboon	0.0 l
DS30199	73.3 de	Kona	6.7 jkl	Fantail	0.0 l
Yukon	70.0 e	Magneta	6.7 jkl	Finwhale	0.0 l
DS30159	53.3 f	Kodiak 1	5.3 jkl	Kiowa	0.0 l
Starfish	53.3 f	PV-1512/Bandera	4.7 jkl	Nenbus	0.0 l
Avenger	41.7 fg	1062	3.7 kl	Patton	0.0 l
Spiros	41.7 fg	Bonobo	3.3 kl	Pheasant	0.0 l
Viroflay 1	36.7 g	Tacoma	2.3 kl	PV-1449/Cocopah	0.0 l
Hammerhead	35.0 g	Dallas	1.7 kl	PV-1526	0.0 l
Whale	35.0 gh	Java	1.3 kl	PV-1599	0.0 l
Viroflay 2	30.0 ghi	Crosstrek	1.0 kl	PV-1610	0.0 l

Minkar	20.0	hij	Tabit	1.0	kl	PV-1611	0.0	l
EXP 3547	16.7	ijk	Bandicoot	0.7	l	PV-1617	0.0	l
Hydrus	15.0	ijkl	Dracus	0.7	l	Regor	0.0	l
Octans	14.3	ijkl	Kodiak 2	0.7	l	Vicuna	0.0	l
El Rio	13.3	jkl	Mykonos	0.7	l	Woodpecker	0.0	l
Bylot	11.7	jkl	Cabazon	0.3	l			
EXP 3546	11.7	jkl	Corvus	0.3	l			

^zDowny 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 ($P \leq 0.05$).