

I. Abstract

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

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Summary:

Downy mildew of spinach continues to be a major production constraint for California. As of March 2012, there are 13 named races of the downy mildew pathogen with isolate UA510C designated as race 13 in 2011. Another deviating strain, UA4410, has appeared in several locations and in multiple years and likely will be designated race 14 in the upcoming year. Downy mildew has been prevalent in many growing areas of California with races 12, 13, and UA4410 being the most common. Also, there have been a number of isolates recovered in 2011 which were a mixture of two or more distinct races. Although it is laborious to sort out race mixtures, we have been able to identify potential mixtures by the initial atypical reactions on the standard set of spinach differentials, followed by inoculum increases on two or more hybrids that have different resistance backgrounds, and subsequent inoculations with the separated isolates. In all cases examined, the initial atypical isolate reactions could be explained by the presence of mixtures of two known races. Several additional atypical isolates have been identified and are currently being examined for their ability to overcome the known resistances. Additional efforts have also looked at the host specificity of the spinach downy mildew pathogen and other closely related downy mildews to determine if the other hosts could serve as a source of primary inoculum to initiate disease on spinach. Thus far, there is no evidence that the spinach downy mildew pathogen can infect other closely related plants; likewise, there is no indication that downy mildew pathogens on plants related to spinach can infect spinach.

II. Main Body of Report

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

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Cooperating Personnel: Numerous seed company personnel, spinach growers, and pest control advisors

Objectives:

1. Maintain a downy mildew race identification service in California and screen contemporary germplasm to predominant races in California.
2. Examine the host range of the spinach downy mildew pathogen and determine if downy mildews on closely related hosts can infect spinach.

Procedures:

Objective 1: Race identifications

Field isolates of the spinach downy mildew pathogen *Peronospora farinosa* f. sp. *spinaciae* (Pfs) are routinely collected from the main spinach production areas in California and Arizona and sent to both the Koike and the Correll labs. The majority of the routine isolate characterization is done in the Koike lab. Any isolates that give unknown or unexpected reactions on the internationally agreed upon differentials are sent to the Correll lab for inoculum increase and further testing to evaluate if the strains are novel or are mixtures of known races. The differentials used for the inoculation and disease evaluation procedures have been selected by Correll and colleagues. A large collection of commercial spinach cultivars, experimental hybrids, and advanced breeding lines are screened with the contemporary races and novel strains to determine which resistance genes and/or combination of resistance genes are the most effective.

Objective 2: Host range

A series of cross-inoculation experiments were conducted to determine if the spinach downy mildew pathogen could infect and cause symptoms on plants closely related to spinach. These other species are all in the *Chenopodium* plant family and include, among others, sugarbeet, beet, Swiss chard, and lambsquarters and nettleleaf goosefoot weeds. In addition, cross inoculation

experiments were conducted to determine if downy mildew isolates from other hosts closely related to spinach could infect and cause symptoms on spinach.

Results and Discussion:

Objective 1: Race identifications

A large collection of isolates was examined for race identity in 2011 using our standard differential series of spinach lines and cultivars (Table 1). Races 11, 12, 13, and UA4410 were identified in multiple locations throughout the year (Fig. 1). Race 13 predominated in most locations likely due to the cultivar selections made for these fields; many of the commercial hybrids planted contained a combination of resistance factors that allowed race 13 to infect the hybrid (Fig. 2). Race 13 was nominated as a named race in 2011 by both the Correll/Koike lab and the International Working Group on *Peronospora* (IWGP). For reference, see the UC Cooperative Extension July/August 2011 issue of Crop Notes or the August 5, 2011 blog posting (<http://ucanr.org/blogs/SalinasValleyAgriculture/>). In the near future it is likely that isolate UA4410 will be designated as race 14.

Some samples were determined to be composed of mixtures of known races. For example, submitted sample UA2511A initially showed an atypical reaction on the differential set. After careful “dissection” of the isolate whereby the original inoculum was increased on spinach lines with different resistances, it was found that the original field isolate was a mixture of races 12 and 13 (Fig. 3).

Two novel strains from the European Union were characterized in 2011 and early in 2012. The isolates were designated as UA4711 and UA1312 and their disease reactions on the differential lines are shown in Table 1. Work is ongoing to further characterize these isolates, determine how prevalent these strains are in commercial spinach production areas, and identify resistance loci that are effective against these deviating strains.

A large collection of spinach cultivars and germplasm was screened for resistance or susceptibility to races 10, 11, 12, and 13 (Table 2). The novel deviating isolate UA4410 was also included in this screening work (Table 2).

Objective 2: Host range

Numerous inoculation tests conducted to date indicate that the spinach downy mildew pathogen, *Peronospora farinosa* f. sp. *spinaciae*, is host specific to spinach and is not able to infect other plants that are closely related to spinach including nettleleaf goosefoot and lambsquarters weeds (Table 3). In addition, the downy mildew pathogen of Swiss chard, *Peronospora farinosa* f. sp. *betae*, a closely related downy mildew, is not able to infect spinach (Table 3). These data are consistent with what has been shown in the literature where it appears that the spinach downy mildew pathogen is host specific and is only able to infect spinach. Therefore, other plant species in the Chenopodiaceae do not serve as a source of inoculum that could initiate disease on spinach.

Publications

Correll, J. C., and Koike, S. T. 2011. Naming of another new race (race Pfs 13) of the spinach downy mildew pathogen. August 5. Salinas Valley Agriculture UCCE blog. <http://ucanr.org/blogs/SalinasValleyAgriculture/>.

Correll, J. C., and Koike, S. T. 2011. Naming of another new race (race Pfs 13) of the spinach downy mildew pathogen. Monterey County Crop Notes newsletter. July/August.

Feng, C., Correll, J. C., Kammeijer, K. E., and Koike, S. T. 2011. Characterization of new races and novel strains of the spinach downy mildew pathogen *Peronospora farinosa* f. sp. *spinaciae*. Abstract and poster. International Spinach Conference. Amsterdam, The Netherlands. October 3-4.

Feng, C., Correll, J. C., Kammeijer, K. E., and Koike, S. T. 2011. Characterization of new races (races 11 and 12) and several novel strains of spinach downy mildew pathogen *Peronospora farinosa* f. sp. *spinaciae*. Abstract. Phytopathology 101:S52.

Koike, S. T., and Correll, J. C. 2012. Spinach downy mildew: preparing for another season. Monterey County Crop Notes newsletter. January/February.

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Table 1. Comparison of two deviating isolates (UA4711 and UA1312) of the spinach downy mildew pathogen from the European Union with standard reaction profiles for races 10 to 13.

Differential	Race						
	10	11	12	13	UA4410	UA4711	UA1312
Viroflay	+	+	+	+	+	+	+
Resistoflay	+	+	+	+	+	+	+
Califlay	+	-	-	+	-	+	-
Polka	+	-	-	+	-	+	-
Bolero	+	+	+	+	+	-	+
Campania	+	-	+	+	+	-	-
Dolphin	+	-	+	-	+	+	-
Avenger	+	-	+	-	+	+	-
Lion	+	-	-	-	-	+	-
Lazio	-	+	+	+	+	-	+
Pigeon	-	-	-	-	+	-	+
NIL1	+	-	+	-	+	+	-
NIL2	-	+	+	+	+	+	+

NIL = near isogenic lines that contain a single resistance locus in an otherwise susceptible background. NIL1 contains the *RPF1* resistance locus and NIL2 contains the *RPF2* resistance locus.

Table 2. Resistance screening of 116 spinach varieties and lines to downy mildew races 10 to 13 and novel strain UA4410.

Cultivar	Race 10	Race 11	Race 12	Race 13	UA4410
Viroflay	+	+	+	+	+
Resistoflay	+	+	+	+	+
Califlay	+	-	-	+	-
Bolero	+	+	+	+	+
Campania	+	-	+	+	+
Avenger	+	-	+	-	+
Lion	+	-	-	-	-
Lazio	-	+	+	+	+
Dolphin	+	-	+	-	+
Polka	+	-	-	+	-
Clermont	+	+	+	+	+
Boeing	+	-	+	-	+
Whale	+	-	-	+	-
501	-	+	+	+	+
505	-	+	+	+	+
692	-	-	-	-	-
80.8373	+	-	-	-	+
80.8393	+	-	-	-	+
80.9448	-	-	-	-	+
8031	+	-	-	+	-
8032	-	-	-	+	-
8078	+	-	-	+	-
8079	-	-	-	+	-
8184	+	-	-	+	-
8185	-	-	-	+	-
51-315	-	-	+/-	-	+
51-703	-	-	+	-	+
51-704	-	-	-	-	+
80-5224	-	-	+	-	+
80-6280	-	-	+	-	+
03-316	+	+	+	+	+
04-103	+	+	+	+	+
88-130	+	+	+	+	+
88-212	+	+	+	+	+
88-310	+	+	+	+	+
91-227	+	+	+	+	+
97-154	+	+	+	+	+
Amazon	-	-	+	-	+
Bahamas	-	-	+	-	+
Bikini	-	+	+	+	+

Bonbini	-	+	+	+	+
C2-606	+	-	-	-	-
Carmel	-	-	+	-	+
Charger	-	-	-	+	-
Clipper	+	-	+	-	+
Cook	-	-	+	-	+
Corfu	-	+	+	+	+
Corvette	-	-	+	-	+
Crescent	+	-	+	-	+
Dolphin	+	-	+	-	+
E80. 6282	-	-	+	-	+
Eagle	+	-	+	+	+
Ebro	-	+	+	+	+
El Forte	-	+	+	+	+
El Grinta	-	+	+	+	+
El Patriot	-	-	-	+/-	-
El Real	-	-	-	+/-	-
Emilia	-	+	+	+	+
Emu	-	+	+	+	+
Fallgreen	+	+	+	+	+
Finch	-	-	-	-	+
Flamingo	-	-	-	+/-	-
FP469	+	-	-	-	-
Fp505	+	-	-	-	-
FS11	+	-	-	-	-
FS41	+	-	-	-	-
FS83	+	-	-	-	-
FZ506	+	-	+	-	+
Giraffe	+	-	+	-	+
Gowan # 1	-	+	+	+	+
Gowan # 2	-	-	+	-	+
Gowan # 3	-	-	-	-	+
Gowan # 4	-	+	+	+	+
Grandi	-	+	+	+	+
Hunter	-	-	+	-	+
Ibiza	-	+	+	+	+
LDSP 930	-	-	-	+/-	-
LDSP 931	-	-	-	+	-
Marabu	-	+	+	+	+
Misano	-	+	+	+	+
Mississippi	-	-	+	-	+
Missouri	-	-	+	-	+
Monza	+	-	+	-	+
NIL1	+	-	+	-	+
NIL2	-	+	+	+	+

O-A	-	-	-	+	-
Ohio	-	+	+	+	+
Ottawa	-	+	+	+	+
Pelican	-	+	+	+	+
Pigeon	-	-	-	-	+
Polar Bear	-	+	+	+	+
PV 0698	-	-	+	-	+
PV-0692/Thames	-	-	-	-	-
PV-7126	-	-	+	-	+
PV-7131	-	-	-	+/-	-
PV-7132	-	-	+	-	+
PV-7136	-	+/-	+/-	+/-	-
PV-7154	-	-	+	-	+
PV-7155	-	-	+	-	+
PV-7156	-	-	+	-	+
PV-7157	-	-	+	-	+
PV9208	-	-	-	+	-
PV9209	-	-	-	+	-
Queen	+	-	+	-	+
Raccoon	-	-	-	+	-
Regal	+	-	+	-	+
Silver Whale	+	-	-	-	-
SPF321	-	-	+	-	+
SPF323	+	-	+	-	+
SPF324	+/-	-	+	-	+
SPF325	+/-	-	+	-	+
SPF326	+/-	-	+	-	+
SPF327	-	-	-	+	-
Squirrel	-	-	+	-	+
Swan	-	-	+	-	+
Tasman	-	+/-	+/-	+/-	+/-
Tbird	-	-	+	-	+
Thames	-	+/-	+/-	+/-	+/-
Tonga	-	-	+	-	+
Tortoiseshell	+	-	+	-	+
Vigore	-	+	+	+	+
VSS91	-	+	+	+	+
VSSC-R1	+	-	+	-	+
VSSC-V1	-	-	+	-	+
Waitiki	-	-	-	+	-
Wallis	-	-	+	-	+
Whale	+	-	-	+	-
Yabi	-	+	+	+	+
Zebu	-	-	-	-	+

Downy mildew disease reaction terminology.

Symbol	Meaning	Explanation
+	Susceptible	$\geq 85\%$ of plants, and often closer to 100%, show symptoms and sporulation on over 50% of individual cotyledons.
-	Resistant	$\leq 15\%$ of plants, and often closer to 0%, show symptoms and sporulation on the cotyledons.
+/-	Segregating	$\geq 15\%$ but $\leq 85\%$ of the plants show symptoms and sporulation on $>50\%$ of individual cotyledons. Often indicative of a segregating line, an impure line, or one with an exceptionally large number of inbred individuals (due to a high degree of selfing from the female line).
+*	Intermediate resistance	$\geq 85\%$ of plants, and often closer to 100%, show symptoms and sporulation but symptoms and sporulation are greatly reduced relative to a susceptible control with less than 50% of the leaf area of individual cotyledons showing symptoms and sporulation including only tip-infections.
+*/-	Intermediate resistance	$\geq 15\%$ but $\leq 85\%$ of the plants show symptoms and sporulation but symptoms and sporulation are greatly reduced relative to a susceptible control with less 50% of the leaf area of individual cotyledons showing symptoms and sporulation including only tip-infections.

Table 3. Chenopodiaceae species used for host range studies of downy mildews from spinach and closely related plants.

Common Name	Botanical Name	Isolates	
		<i>P. farinosa</i> from spinach	<i>P. farinosa</i> from Swiss chard
Edible Chenopods			
Spinach	<i>Spinacia oleracea</i>	+	-
Table beet	<i>Beta vulgaris</i>	?	+
Sugar beet R	<i>Beta vulgaris</i>	?	-
Sugar beet S	<i>Beta vulgaris</i>	?	+
Swiss chard	<i>Beta vulgaris</i> subsp. <i>cicla</i>	?	+
French spinach (orach)	<i>Atriplex hortensis</i>	?	?
Quinoa	<i>Chenopodium</i> <i>quinoa</i>	?	?
Huazontle	<i>Chenopodium</i> <i>nuttalliae</i>	?	?
Epazote	<i>Chenopodium</i> <i>ambrosioides</i>	?	?
Beetberry	<i>Chenopodium</i> <i>capitatum</i>	-	?
Spinach Relatives			
Undomesticated spinach	<i>Spinacia</i> <i>turkestanica</i>	+	?
	<i>Spinacia tetrandia</i>	?	?
	<i>Monolepis</i> <i>nuttalliana</i>	?	?
Weed Chenopods			
Prostate knotweed	<i>Polygonum</i> <i>aviculare</i>	?	?
Lambsquarter	<i>Chenopodium album</i>	-	?
Nettleleaf goosefoot	<i>Chenopodium</i> <i>murale</i>	-	?

? = not tested.

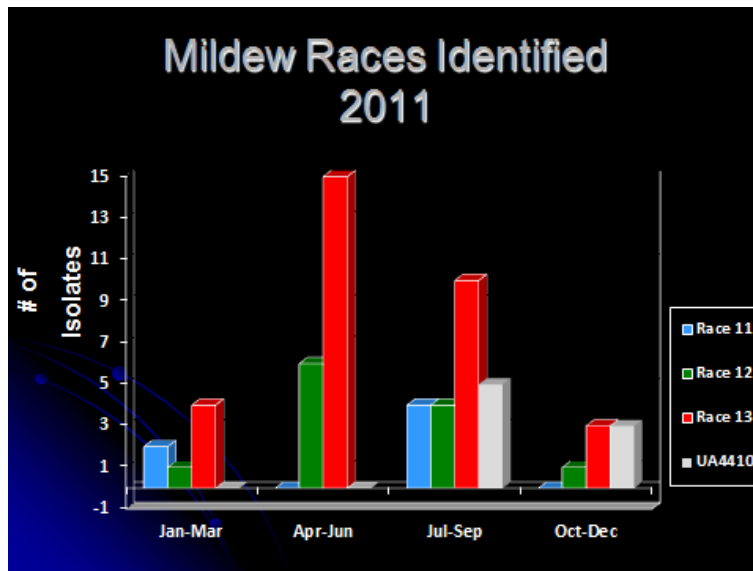


Figure 1. Frequency of races of the spinach downy mildew pathogen from California samples in 2011.

Many hybrid cultivars

RPF2 x *RPF3*
Susceptible to race 13

Resistant to races	Resistance locus	
	<i>RPF2</i>	<i>RPF3</i>
	1-10	1,3,5,8,9,11,12,"14"
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
UA4410		
Super Race?		

Designation	Expected Disease Reaction
	Susceptible
	Resistant

Figure 2. Example of a hybrid spinach susceptible to race 13.

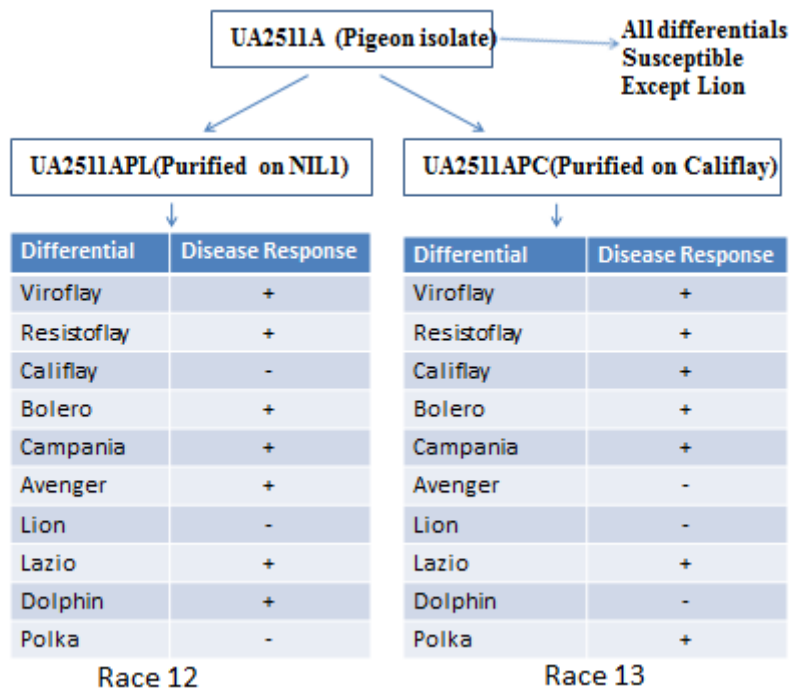


Figure 3. Example of isolates collected as “mixtures” and how the combination of races is determined.