Update on downy mildew and white rust on spinach in the United States

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Abstract: Downy mildew, caused by *Peronospora farinosa* f. sp. *spinaciae*, and white rust, caused by *Albugo occidentalis*, are economically important diseases of spinach. Downy mildew disease is important throughout the world where ever spinach is grown whereas white rust is an important disease in spinach production areas of the central and eastern United States. Our efforts over the years have focused on improved management of these two diseases primarily through improved disease resistance, fungicides, and fungicide alternatives. In addition, efforts have continued to characterise races of the downy mildew pathogen and develop efficient screening methods to identify resistance. Screening for disease resistance has involved field, traditional greenhouse assays, and modified greenhouse assays. Recent efforts have identified three new races of the downy mildew pathogen, races 5, 6, and 7. Although a qualitative inoculation assay has traditionally been used to identify resistance to the various downy mildew races, work with race 6 indicates that a modified assay can identify both qualitative (+ or -) and quantitative resistance to race 6. As a result, to better communicate the level of resistance in a particular cultivar, it is recommended that resistant (or immune) be used when cotyledons or true leaves do not get infected and show symptoms, tolerant, or partially resistant, be used if cotyledons are susceptible, but true leaves show quantitative levels of resistance, or susceptible, if both cotyledons and true leaves are susceptible. Quantitative levels of resistance also are commercially useful to manage white rust disease of spinach. By selection under heavy disease pressure, the University of Arkansas program has released a number of spinach lines with high levels of commercially useful resistance to this disease. In addition to resistance, judicious use of fungicides can enhance control of both of these diseases. In addition, we have identified a number of surfactants and natural products that have been effective at reducing white rust and downy mildew severity.

Introduction

Spinach (*Spinacia oleracea*) is an important vegetable crop grown on more than 17,000 hectares in the United States and is valued at approximately 184 million dollars annually (Lucier, 2002)). Major areas of production include California, Colorado, Texas, Arkansas, Oklahoma and parts of the east coast (Correll et al., 1994). Downy mildew, or blue mold, caused by *Peronospora farinosa* (Fr.) Fr. f. sp. *spinaciae* Byford (Brandenberger et al, 1991), is an economically important pathogen on spinach in most regions where the crop is grown. Prior to 1996, four races of the pathogen were known to occur in the U. S. and Europe (Table 1). Although major (qualitative) and minor (quantitative) gene resistance to the various downy mildew races has been identified in spinach (Brandenberger et al. 1991; Brandenberger et al. 1994; Correll, 1998; Eenink, 1976; Irish et al. 2003; Jones and Dainello, 1982), major gene resistance has been the type of resistance used almost exclusively to manage this disease (Correll, 1998). Screening for major gene resistance to *P. farinosa* f. sp. *spinaciae* has typically relied on a qualitative cotyledon greenhouse infection assay and has become the routine industry standard for evaluating resistance.

Beginning in 1996, cultivars with resistance to the four previously known races (races 1, 2, 3 and 4) of the pathogen were observed with high levels of disease in commercial fields in California and Europe. Consequently, work was initiated to document if new races of the pathogen were present based on disease reactions on differential cultivars; a survey of prevalent races in California also was conducted. In addition, commercial cultivars were

screened for qualitative and quantitative resistance to two new races (race 5 and 6) of the pathogen common in California.

Table 1. History of the occurrence of races of *Peronospora farinosa* f. sp. *spinaciae*

Race	Year[a]	Location
Race 1[b]	1824	U.S./Europe
Race 2	1958	California
	1958	Europe
Race 3	1976	Netherlands
	1978	California
	1982	Texas
Race 4	1990	California
	1990	Japan
	1991	California
	1991	Texas
	1994	Europe
Race 5	1996	Colorado
	1997	California
	1998	Europe
	1998	Europe
	2002	Japan
Race 6	1998	California
Race 7	1999	Europe

[a] Year the race of *P. farinosa* f. sp. *spinaciae* was observed or reported.
[b] The year the pathogen was observed and described and later assigned a race 1 designation.

Materials and methods

Planting and plant maintenance

Spinach seed (without fungicide treatment) was sown directly into Redi Earth Mix (Scott-Sierra, Marysville, OH) in flats with 10 rows and 20 seed per row. Plants were grown in a greenhouse with temperatures ranging from 15-25^{O}C. Plants were watered daily and fertilized once a week beginning 7 days after planting with an all-purpose fertilizer (Peters 20/20/20).

Race identification tests were conducted as previously described (Brandenberger et al, 1991; Irish et al, 2003). Seedlings (7-10 day old) were inoculated at the cotyledon stage, incubated at 100% RH and 20^{O}C for 24 hrs, and then incubated in a growth chamber maintained at 20^{O}C with a 12-hr light/dark cycle for six days. Six days after inoculation, plants were returned to the dew chamber maintained at 20^{O}C and 100% relative humidity for 18-24 hrs.

To quantify resistance to races 5 and 6, cotyledons and the first set of true leaves on two-week old seedlings were inoculated. The first set of true leaves were approximately 2 cm long at the time of inoculation.

For race identification, cotyledons were evaluated qualitatively for sporulation of the pathogen 18-24 hrs after the dew chamber treatment to induce sporulation. Any genotype with sporulation on > 85% of the total number of cotyledons was considered susceptible whereas any genotype with sporulation on < 15% of the cotyledons was considered resistant as previously described. Genotypes with intermediate reactions also were recorded.

To quantify resistance to race 5 and race 6, both the cotyledons and the true leaves were evaluated for disease reactions. After sporulation was induced, each cotyledon was scored for

the presence or absence of sporulation as previously described. The true leaves also were evaluated on a scale of 0-4 based on the percentage leaf area with symptoms and sporulating lesions; with 0 = no signs or symptoms of infection; 1 = 1-25% of the leaf area with sporulation and symptoms; 2 = 26-50%; 3 = 51-75%; and 4 = >75%.

Table 2. Disease reactions of differential spinach genotypes used to characterize races of the spinach downy mildew pathogen, *Peronospora farinosa* f. sp. *spinaciae*).

		Race[b]		
Genotype[a]	Resistance	race 5	race 6	race 7
Viroflay	none	+	+	+
99 X 95	1,2	+	+	+
Califlay	1,3,5	-	+	+
Polka	1,2,3,5	-	+	+
Bolero	1,2,3,4	+	+	+
Whitney	1,2,3,4	+	+	+
Rushmore	1,2,3,5	-	+	+
Campania (SAS1)	1,2,3,4,5,7	-	+	-
Lion	1,2,3,4,5,6,7	-	-	-
St. Helens	1,2,3,5	-	+	+
NAKG1 (Winterreuzen)	none	+	+	+
NAKG2 (Nores)	1,2	+	+	+
NAKG3 (Califlay)	1,3,5	-	+	+
NAKG4 (Polka)	1,2,3,5	-	+	+
NAKG5 (Rushmore)	1,3,4,5	-	+	+
NAKG6 (Bolero)	1,2,3,4	+	+	+
NAKG7 (Spinnaker)	1,2,3,4,5	-	+	+
NAKG8 (Spicer)	1,2,3,4	+	+	+
NAKG9 (San Felix)	1,2,3,4,5	-	+	+
NAKG10 (Clermont)	1,2,3,4	+	+	+
NAKG11 (Lion)	1,2,3,4,5,6,7	-	-	-
NAKG12 (Scenic)	1,2,3,4,5,7		+	
NAKG13 (El Dorado)	1,2,3,4,5,7	-	+/-	-
NAKG14 (Eagle)	1,2,3,4?	+	+/-	+/-

[a]Named or numbered genotypes. Parenthetical designations are alternative names or commercial cultivar names also used with the numbered genotypes.

[b] Race designations were based on the disease reaction of the differential genotypes. A "+" indicates that > 85% of the cotyledons showed evidence of infection and sporulation after 7 days and were considered susceptible. A "-" indicates that <15% of the cotyledons showed evidence of infection and sporulation after 7 days and were considered resistant. A "+/- indicates an intermediate reaction.

Results and discussion

A standard nomenclature for designating races of the spinach downy mildew pathogen, *P. farinosa* f. sp. *spinaciae*, is needed in order to accurately document the resistance of cultivars. Documentation of the resistance also is important for seed companies to release and promote cultivars. Three new races of *P. farinosa* f. sp. *spinaciae*, designated races 5, 6, and 7, have been identified.

Table 3. Quantitative differences in resistance to race 6 of *Peronospora farinosa* f. sp. *spinaciae*

		Cotyledons[b]		True leaves[cd]		
Cultivar	NAKG[a]	Test 1	Test 2	Test 1	Test 2	Rank[e]
Winterreuzen	NAKG1	+	+	4.0 a	3.9 a	HS
Clermont	NAKG10	+	+	4.0 a	3.8 a	HS
Viroflay		+	+	4.0 a	3.7 a	HS
Spicer	NAKG8	+	+	4.0 a	3.7 a	HS
Whitney		+	+	3.1 cd	3.0 b	HS
Bolero		+	+	3.4 ab	1.3 c	S
Bolero	NAKG6	+	+	2.1 de	1.3 c	S
Califlay		+	+	2.6 cd	1.1 cd	S
Nordic IV (SanFelix)	NAKG9	+	+	2.0 def	1.0 cd	S
Califlay	NAKG3	+	+	2.6 cd	1.0 cd	S
Rushmore		+	+	1.6 efg	1.0 d	S
Campania		+	+	2.8 bc	0.9 de	S
Spinnaker	NAKG7	+	+	1.1 gh	0.7 ef	MR
Rushmore	NAKG5	+	+	1.6 efg	0.5 fg	MR
	NAKG14	+	+	1.0 gh	0.5 fg	MR
Polka		+	+	0.9 gh	0.5 fg	MR
Nores	NAKG2	+	+	1.4 fgh	0.5 fg	MR
Polka	NAKG4	+	+	1.0 gh	0.4 fg	MR
99 x 95		+	+	0.9 h	0.3 gh	MR
SG6207 (El Dorado)	NAKG13	+/- (61)[f]	+/- (27)[f]	0.1 i	0.1 hi	MR
Lion		-	-	0.0 i	0.0 i	R

[a] European germplasm source.
[b] A "+"= >85% of cotyledons were susceptible. A "-" indicates that <15% of the seedlings showed evidence of infection and sporulation after 7 days. A "+/- indicates an intermediate reaction.
[c] True leaves were rated on a scale of 0-4 where 0 = no symptoms; 1 = 1-25% disease; 2 = 26-50%; 3 = 51-75%; and 4 = 76-100%.
[d] Numbers followed by the same letter within a column are not significantly different from one another ($P = 0.05$).
[e] Disease reaction categories: HS = highly susceptible; S = susceptible; MR = moderately resistant; R = resistant (or immune).
[f] Percentage of cotyledons that were susceptible.

Historically, screening for downy mildew resistance has relied on a qualitative disease assessment on the cotyledons of spinach seedlings. The absence of symptoms and sporulation indicated, albeit often presumptively, that a given genotype had resistance governed by a major (qualitative) resistance gene. When commercial breeders screen spinach germplasm to identify resistance to a new race of the downy mildew pathogen, the resistance either is incorporated into open pollinated cultivars or, more often, into hybrids. Consequently, the resistance used to a particular race by the various seed companies may not necessarily be associated with the same resistance gene (ie. the resistance may not be allellic). This scenario was apparently the case for spinach cultivars with resistance to race 5. Although many of the spinach cultivars grown in California had resistance to races 1, 2, 3, and 4, some of these cultivars proved to be susceptible to race 5 while others proved to be resistant. When the parentage of these cultivars was examined, it was apparent that there were two different genes (or alleles) from different sources governing resistance to races 1 and 3, one of which imparted resistance to race 5. Alternatively, the source of resistance to races 1 and 3 was closely linked to the resistance gene for race 5 resistance (Correll, *unpublished*). This alternate

gene or allele (or linked resistance gene) appeared to be present in some of the cultivars evaluated and was effective against race 5. "

The appearance of race 6 in California was particularly problematic in that none of the commercial spinach cultivars evaluated displayed any qualitative resistance to this race at the time this new race appeared. All commercial cultivars were rated as susceptible to race 6 based on the cotyledon infection assay. However, field observations indicated that the cultivars considered susceptible to race 6 based on the greenhouse cotyledon assay exhibited quantitative differences in resistance to downy mildew under field conditions (Schafer and Correll, unpublished). Consequently, a greenhouse true-leaf assay was developed to evaluate quantitative differences in susceptibility by inoculation of true leaves. These results clearly indicated significant quantitative differences in resistance among cultivars. Although the correlation between greenhouse observations of quantitative resistance and field reactions still needs to be evaluated further, field observations to date have been consistent with the greenhouse tests. Also of interest, the original male and female inbred parents of one particular hybrid were susceptible to race 6 in a cotyledon assay whereas the hybrid itself was intermediate in its reaction and showed a substantially higher level of resistance at the cotyledon stage than either of the original parents. Thus, little is known of the actual genetic control of resistance to *P. farinosa* f. sp. *spinaciae*.

Future research efforts on downy mildew will need to focus on monitoring and characterizing new races of the pathogen. Because most commercial spinach cultivars with different resistance genes are hybrids, it is important for the long term that a set of open-pollinated isogenic differentials be developed that contain different resistance genes. In addition, breeding efforts should continue to evaluate and utilize quantitative resistance to this important disease.

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