Occurrence of Race 3 of *Peronospora effusa* on Spinach in Texas and Identification of Sources of Resistance

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ABSTRACT

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Race 3 of *Peronospora effusa* capable of attacking spinach (*Spinacia oleracea*) resistant to races 1 and 2 has been found in Texas. Race 3 is currently causing severe damage on fresh market savoy spinach. A source of resistance based on the gene designated M_3 has been identified in some breeding lines and commercially available hybrids. A technique suitable for resistance screening is described.

Additional key words: blue mold, downy mildew, pathogen variability

Downy mildew or blue mold of spinach (*Spinacia oleracea* L.) incited by *Peronospora effusa* (Grev. ex Desm.) (syn. *P. spinaciae* Laub. and *P. farinosa* Fr.) (13,15) is potentially the most devastating disease of this crop in Texas and other states (8). Annually, Texas leads the nation in spinach production with approximately 4,047 ha valued at $10–15 million (1,12). Ninety percent of the Texas acreage, or one-third of the U.S. crop, is produced in three counties (Frio, Uvalde, and Zavala) of the Wintergarden region.

Genetically resistant hybrids have been used to control blue mold in spinach. At least two race-specific genes confer resistance in spinach to designated races 1 and 2 of *P. effusa*. Race 1 was observed in Texas and California in the 1950s (10,14). At that time, blue mold seriously threatened the spinach industry in the Wintergarden region. Resistance to race 1 was identified by Smith in PI 140467 (9). This material, collected from Iran, was demonstrated to contain a dominant gene for resistance. Califlay, a new cultivar of spinach resistant to race 1 of *P. effusa*, was derived by crossing PI 140467 × Viroflay, followed by four backcrosses to Viroflay (11).

In 1947, H. A. Jones began an F_1 hybrid program to develop cultivars adapted to the Wintergarden region of Texas (5,14). This program utilized the USDA line 99x95 as the source of blue mold resistance and resulted in the release of slow bolting, downy-mildew-resistant hybrids such as 612, 621, Dixie Market, Early Hybrid 7, and EH 424. The genetic resistance conferred by these hybrids was stable and virtually eliminated blue mold as a significant production problem in Texas for more than 20 yr.

In 1958, blue mold was observed on commercial plantings of Califlay in the Salinas Valley of California. Test inoculations confirmed that a second physiologic race (designated race 2) had developed (16). Smith et al (10) showed that whereas Califlay and its derivatives had resistance only to race 1 (conferred by the single dominant gene designated M_1), other USDA breeding lines (including 99x95, the seed parent of Early Hybrid 7) possessed effective resistance to both race 1 and race 2. This latter gene, designated M_2 by Smith et al (10), actually consisted of two closely linked, race-specific genes (2,3) (hereinafter referred to as M_1,M_2). Krober et al (6) confirmed the reaction of Califlay as resistant to race 1 but susceptible to race 2.

In 1975, Eenink observed a third race of *P. effusa* in the Netherlands (4). Two years later, blue mold was observed in California and Texas on cultivars containing M_1,M_2 resistance. Since 1977, blue mold has been observed with increasing frequency on hybrids resistant to races 1 and 2. In 1980 and 1981, blue mold reached near epiphytotic proportions in Texas, with an estimated yield loss of 20 and 30%, respectively. Severe disease has been observed during this 2-yr period on each of the six major fresh-market hybrids grown in the Wintergarden region. Fields were frequently identified with more than 60% of the plants exhibiting chlorosis and typical sporulation of the blue mold fungus. All fields examined were planted to hybrids reported to contain M_1,M_2 type resistance to *P. effusa*.

This report confirms the presence of race 3 of *P. effusa* in Texas, which is virulent on hybrids containing resistance to races 1 and 2 but is not virulent on differential lines containing resistance to races 1 and 3 or races 1, 2, and 3 of this organism. A nomenclature system further extending that suggested by Smith et al (10) is proposed.

MATERIALS AND METHODS

Source of isolates. Two isolates were collected from leaves containing spores in separate fields. Freshly collected material is superior to stored material for obtaining a high percentage of germinating conidia (7). One isolate (TAES) was collected from sporulating lesions on the hybrid Iron Duke at the Texas Agricultural Research and Extension Center, Uvalde. The Uvalde isolate was collected from sporulating lesions on Hybrid 621 on the John Miyakawa Farms, Uvalde. The hybrids Iron Duke and 621 contain M_1,M_2 resistance.

Inoculation tests. Each isolate was used in separate trials to inoculate two distinct sets of spinach differential cultivars or lines (Table 1). Seeds were soaked in deionized water for 24 hr at 12 C, drained, and incubated an additional 24 hr at 12 C to promote germination. Germinating seeds were then sown in Jiffy 7 peat pellets (10 seeds per pellet, six pellets per differential) and placed under light banks that provided 3,300 lux at 53 cm. Approximately 14 days after sowing, cotyledons and primary leaves of plants in each pellet were sprayed with 0.5 ml of a suspension containing 3×10^4 conidia per milliliter. Conidial suspensions were prepared by wet-brushing spores from leaves with a camel hair brush, quantifying with a hemacytometer, and adjusting the spore concentration with deionized water. Flats were inoculated at 1600 hr on each of 3 consecutive days and incubated in a growth chamber at 10 C under the artificial lighting in a 9/15 day/night regime to prevent bolting. Humidity was maintained with a cool-vapor humidifier to provide leaf wetness during the nighttime hours.

Plants were misted intermittently for 16 hr following each inoculation and then incubated for 5 days at 20 C in a greenhouse. Following incubation, plants were placed back in the chamber and rehumidified for 3 days. Plants were examined for visible sporulation at 0900 hr each day. Infected plants were counted and removed as they were identified.

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Table 1. Incidence of blue mold on seedlings of differential lines of spinach inoculated with two isolates of *Peronospora effusa* from Texas

Cultivar or line		Resistance genes	Blue mold incidence[a]		Mean infection (%)	Reaction[b]
Set 1	Set 2		TAES isolate	Uvalde isolate		
Sakata		None	31/32	44/46	96.2	S
	DMCO3	None	59/60	38/39	98.0	S
Ozarka		M_1,M_2	32/35	36/41	89.5	S
	DMCO4	M_1,M_2	20/24	27/29	88.7	S
Hy 62		M_1,M_3	2/37	0/38	2.7	R
Califlay		M_1,M_3	0/39	0/49	0.0	R
	DMCO1	M_1,M_3	0/51	0/54	0.0	R
St. Helens		M_1,M_2,M_3	0/31	1/30	1.6	R
Chinook		M_1,M_2,M_3	1/33	0/36	1.4	R
	DMCO2	M_1,M_2,M_3	1/36	0/38	1.4	R

[a] Number of seedlings exhibiting sporulation of *P. effusa*/ number evaluated. Numbers of seedling evaluated in each genotype varied because of differences in seedling emergence and establishment.
[b] Reaction of >85% considered susceptible and of <5% considered resistant.

RESULTS AND DISCUSSION

Spinach genotypes similar to those grown commercially in the Wintergarden region were susceptible to both isolates of *P. effusa* examined in this study (Table 1). A genotype was considered susceptible when a high percentage of seedlings (>85%) exhibited sporulation of *P. effusa* on cotyledons and primary leaves and resistant when a low percentage of seedlings (<5%) exhibited sporulation. Several cultivars and lines were resistant to both isolates of *P. effusa* studied.

Disease surveys conducted in 1980 indicate that this new race of *P. effusa* poses a serious threat to the spinach industry in southern Texas. Trial plantings of hybrid Chinook (M_1,M_2,M_3) were established at 10 locations throughout the Wintergarden region in 1981. All plantings were interspersed among commonly grown cultivars with M_1 and M_2 genes for resistance. Blue mold developed extensively in six of 10 of these fields, and in all cases Chinook remained free of disease.

The similarity in differential reaction of the new race on the cultivar Califlay to the race 3 reported by Eenink (4) when viewed in light of the chronology of appearance suggests that race 3 may have originated in the Netherlands and subsequently been introduced into the United States on seed or plant parts.

Although the damage to spinach resulting from proliferation of race 3 is great, it is encouraging that a source of resistance has been identified. Although currently available sources resistant to race 3 are not acceptable in Texas for fresh market production because they lack the dark green color and savoy characteristics preferred by our markets, these attributes could be developed in a modest breeding program.

Because the blue mold pathogen has demonstrated physiologic specialization, new races of *P. effusa* may develop as resistance genes are deployed. New sources of resistance should be sought, and the linkage relationship between genes conditioning resistance to race 1 and race 3 in Califlay needs to be examined. We offer the proposed method of gene designation (M_1,M_2,M_3) as an extension of the system proposed by Smith et al (10) in hopes that it will clarify the host-pathogen combinations that are now known to exist between spinach and *P. effusa*.

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