Fusarium oxysporum f. sp. *pisi*, Race 6: Occurrence and Distribution

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ABSTRACT

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A new race of *Fusarium oxysporum* f. sp. *pisi* was isolated repeatedly from commercial pea-growing areas in western Washington. This race was first observed in 1971 and by 1977 had been recovered from 175 of 640 fields examined. This pathogen was detected either in fields previously free of visible wilt symptoms or in fields with a low incidence of race 2. The symptoms and the cultural morphology of the pathogen are identical to those of races 1 and 5. Race 6 can be distinguished from races 1, 2, and 5 by the reaction of six differential cultivars including New Season, WSU 23, and WSU 28. This pathogen meets all requirements of being classified as a new race of pea wilt in that (i) it is widespread, (ii) host resistance is inherited by a single dominant gene, and (iii) pathogenicity tests using differential cultivars with single gene markers for resistance or susceptibility to races 1, 2, and 5 are identical with field reactions. Therefore it is proposed that this new strain be designated race 6.

Wilt of peas (*Pisum sativum* L.), caused by *Fusarium oxysporum* Schlecht. f. sp. *pisi* (van Hall) Snyd & Hans., continues to be a serious economic threat to pea production in the Pacific Northwest (3,4,7,8,10,11). Of 11 races of *F. oxysporum* f. sp. *pisi* previously described (1,2,4,11,12,14,15), only races 1, 2, and 5 are considered to be valid (6, 9). In addition, resistance of pea cultivars to races 1, 2, and 5 are determined by single, separate dominant genes in the host (3–5,9,16,17).

In 1971, many similar isolates of *F. oxysporum* f. sp. *pisi* were isolated from wilted plants collected from a severely infested pea field near Snohomish, WA. These isolates killed pea cultivars and PI accessions resistant to races 1, 2, and 5. This article reports the geographic distribution of this new race and proposes that it be classified as race 6, based on its prevalence in nature, specific pathogenicity, and the genetics of host resistance.

MATERIALS AND METHODS

Geographic distribution. Geographic occurrence of this new race of *F. oxysporum* f. sp. *pisi* was determined by direct isolation from wilted plants and by collecting soil from infested pea fields.

Plants suspected of being infected with *F. oxysporum* f. sp. *pisi* were collected from areas of the field in which most plants exhibited disease symptoms. Plant material was surface disinfested with 1% sodium hypochloride for 2 min and plated on acidified potato dextrose agar (PDA), pH 4.5. Six 5–7 mm stem sections were cultured from each test plant. The cultures were then incubated under cool white fluorescent light, 12-hr photoperiod, for 5–7 days. Small agar plugs were removed from the margins of resulting colonies, placed in sterile Kerr's medium, and incubated on a reciprocal shaker.

Soil samples, approximately 4 L, were collected only from those areas of the field suspected of being infested with *F. oxysporum* f. sp. *pisi*. In all instances, soil was collected from that area of the field in which the disease was the most severe as determined by symptoms on the pea plants. No attempt was made to grid-sample fields. Samples were collected only to determine the race of the fungus present, not the distribution of the fungus within specific fields. The soil was first screened through a 6-mm screen, then treated with 40 ppm of Dexon (p-[dimethylamino] benzenediazo sodium sulfonate) to reduce populations of phycomycetes, and placed in 15.2-cm plastic pots. Ten seeds of each of the six differential pea cultivars listed in Table 1 were planted in each pot. Resultant seedlings were grown for 30–40 days at greenhouse temperatures of 20–24 C, after which they were examined for wilt symptoms. Wilt symptoms consisted of stunting, yellowing, dying of lower leaves, and downward curling of leaf margins, which usually resulted in death of the plant.

Isolate characteristics and inoculum buildup. The predominant "wild type" culture recovered from field-grown, wilted plants or from plants grown in soil collected from these fields was a white, restricted colony with aerial mycelium and little or no conidial production on PDA. All cultures were single spored on 2% water agar, increased on fresh PDA under cool white fluorescent lights with a 12-hr photoperiod. Light intensity ranged from 250 to 300 fc at culture surface. Only colonies representative of the wild type were maintained in sterile soil tubes (4,9).

Inoculum of each test isolate was produced in liquid culture according to procedures described previously (4,9). The spore concentration of each isolate was determined with a hemocytometer and adjusted to 5×10^6 conidia per cubic centimeter.

Inoculation and incubation. The pathogenicity of each isolate was determined by inoculating each of the differential cultivars (Table 1) using the root prune and dip technique (6,13,16,17). When seedlings grown in vermiculite were 10 days old and had produced 4–5 nodes, they were inoculated as follows: Plants were pulled from vermiculite, and the roots were cut at approximately 4 cm below the cotyledon attachment, dipped into the inoculum to cover the cotyledons, transplanted into sterile perlite, and watered with a nutrient solution containing 100 ppm of N, P, and K plus micronutrients. Greenhouse temperatures were maintained at

TABLE 1. Reaction of differential pea varieties to four races of *Fusarium oxysporum* f. *pisi*

Cultivar	Races of wilt			
	1	2	5	6
Little Marvel	32[a](S)[b]	32 (S)	32 (S)	32 (S)
Darkskin Perfection	1 (R)	32 (S)	32 (S)	32 (S)
New Era	0 (R)	1 (R)	32 (S)	32 (S)
New Season	0 (R)	1 (R)	32 (S)	1 (R)
WSU 23	1 (R)	2 (R)	0 (R)	31 (S)
WSU 28	3 (R)	30 (S)	0 (R)	1 (R)

[a]Number of pea seedlings killed of 32 inoculated seedlings, four tests of eight seedlings per test.
[b]Reaction of variety or specific genes for resistance. R = resistant. S = susceptible.

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20–24 C, and all plants were grown under available light. The susceptible cultivars died 21–28 days after inoculation.

Inheritance of host resistance. To determine the genetic basis for resistance to this new race of *F. oxysporum* f. sp. *pisi*, the cultivar Little Marvel (susceptible to races 1, 2, 5, and 6) was crossed with PI accession 244092 (resistant to races 1, 2, 5, and 6) and PI 206780 (susceptible to races 1 and 2 and resistant to race 5 and 6). The F_1 and F_2 populations were tested for resistance to races 1, 2, 5, and 6.

RESULTS

The symptomology of this new race of *F. oxysporum* f. sp. *pisi* is similar, if not identical, to that of races 1 and 5 (4,7,8,10,11). Wilt symptoms were first observed in small areas in a field, and with intensive culture of peas, the fungus may spread throughout the entire field in 1–2 yr.

This new race was first detected in 1971 near Snohomish and by 1974 occurred in the western Washington counties of Snohomish, Skagit, Whatcom, Island, and Grays Harbor. The frequency of occurrence of this new race, from 1973 through 1977, is presented in Table 2. Bioassays for *F. oxysporum* f. sp. *pisi* in 640 fields showed that 27% of the fields were infested with race 6 and 40% with race 5. Because the current differential varieties do not separate mixtures of races 2 and 6 or 5 and 6 from potential new races of *F. oxysporum* f. sp. *pisi*, 4% of the fields were classified as unknown.

The wilt reaction of six differential cultivars with known single dominant genes for resistance to races 1, 2, and 5 were compared with the wilt reaction caused by this new race (Table 1). The differential cultivars New Season and WSU 28 distinguished this new race. Typical wilt reactions on the differential varieties are illustrated in Fig. 1. The pathogenicity of races to specific differentials was constant, and the typical reaction of a virulent culture to a susceptible differential was death of plants of that differential in 21–28 days after inoculation. Virulence of isolates varied, and in some instances isolates of specific races did not kill the susceptible differential variety in 21–28 days. However, the reaction of the differential cultivars remained constant with respect to resistance or susceptibility.

All F_1 plants from the crosses of Little Marvel × PI 244092 and Little Marvel × PI 206798 were resistant and the F_2 progeny segregated in the expected ratio of 3:1, resistant to susceptible, as demonstrated by a χ^2 of 0.027 (Table 3). This meant that a single dominant gene factor was responsible for the resistance. Additional studies of the race 6-resistant cultivars New Season, WSU 28, Aspin, and Grant confirmed that resistance to race 6 was inherited by a single dominant gene.

DISCUSSION

The criteria we used to describe race 6 as a new and distinct type of *F. oxysporum* f. sp. *pisi* are consistent with the methods and techniques used to describe races 1, 2, and 5. Races 1, 2, 5, and 6 are the only races of Fusarium wilt of peas known to occur in nature at levels sufficient to be of economic importance. The concepts of race classification and methods of race identification of *F. oxysporum* f. sp. *pisi* have been discussed (3,6,9). We consider races 3, 4, and 6–11 as described by Schreuder, Bolton et al, and Armstrong and Armstrong (1,2,12) to be isolates of races 1 and 2, varying only in levels of virulence.

Tables 2 and 3 and Fig. 1 demonstrate that the pathogenicity of races 1, 2, 5, and 6 of *F. oxysporum* f. sp. *pisi* can be distinguished by their reaction on the six differential varieties. The reaction of the differentials to these specific isolates of *F. oxysporum* f. sp. *pisi* is not based on variation in symptom severity but on resistant (no observable disease) and susceptible (dead plant) reactions. Such resistant and susceptible reaction of the differential varieties also was observed under field conditions and when field soil was assayed in the greenhouse.

Use of differential varieties with known single dominant genes for resistance to *F. oxysporum* f. sp. *pisi* is essential. The differentials must be maintained as selected pure lines of the variety and not obtained on the commercial market. For example, seed lots of New Season have been tested that were susceptible, segregating for resistance and resistant to race 6. The differentials used in these studies were selected pure lines of the indicated variety that may not necessarily have the same wilt reactions as the commercial variety sold under the same name. Because of this and the need for uniformity in wilt identification, limited seed stock of the indicated differentials is available from the authors for use in the identification of races of *F. oxysporum* f. sp. *pisi*.

TABLE 2. *Fusarium oxysporum* f. sp. *pisi* races in western Washington

	No. of soil[a] samples assayed	Race of wilt identified from sample[b]					
		No wilt	Race 1	Race 2	Race 5	Race 6	Unknown[c]
1973	154	26	...	1	77	50	...
1974	147	20	...	2	77	48	...
1975	141	52	1	1	50	34	3
1976	136	56	3	...	38	21	18
1977	62	21	...	...	16	20	5
Total	640	175	4	4	258	173	26
% Incidence		27.4	0.6	0.6	40.3	27	4.1

[a]Field samples collected by growers and fieldmen from area of suspected infestation with Fusarium wilt.
[b]Number of samples in which specific wilt was detected.
[c]Soils in which the reactions of the differential varieties were atypical, indicating mixtures of wilt races or new races of Fusarium wilt.

TABLE 3. Wilt reaction of F_2 progeny of crosses between resistant Plant Introduction (PI) selections and susceptible Little Marvel

Line or Variety	Wilt reaction of 24 inoculated seedlings[a]			
	Race 1	Race 2	Race 5	Race 6
PI 244092	0/24	0/24	0/24	0/24
PI 206798	24/0	18/6[b]	0/24	0/24
Little Marvel	24/0	24/0	24/0	24/0
F_2 244092 × PI 206798	8/16	6/18	0/24	0/24
F_2 LM × 244092	5/19	6/18	8/16	6/18
F_2 Little Marvel × 206798	24/0	20/4[b]	6/18	5/19

[a]The number of seedlings susceptible/resistant (dead/live) after inoculation with 5×10^5 concentration of conidia by the dip-cut technique.
[b]PI 206798. Seed lot was segregating for resistance to race 2 of *F. oxysporum* f. sp. *pisi*.

The requirement for accurate classification and identification of wilt races can best be illustrated by the necessity of pea growers and processors to index specific fields infested with *F. oxysporum* f. sp. *pisi*. As indicated in Table 2, more than 640 fields were indexed for possible fungus infestation by 1977. Indexing is necessary in order for growers to select the resistant cultivar to be planted in that specific location. Currently, commercial varieties resistant to races 1, 2, 6; 1, 5; 1, 6; and 1, 5, and 6 are available to growers in western Washington. Races 5 and 6 are the limiting factor in pea production on an estimated 10,000 and 5,000 acres of land, respectively, in northwest Washington.

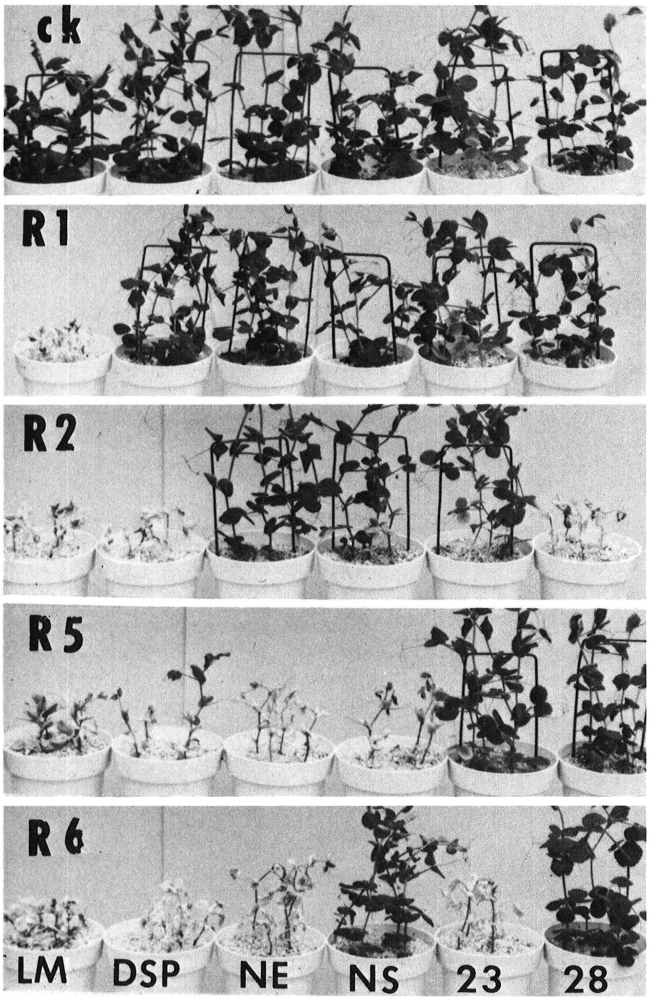

Fig. 1. Pathogenicity of four races of *F. oxysporum* f. sp. *pisi* on six differential varieties of peas: Little Marvel (**LM**), no dominant genes for resistance; Darkskin Perfection (**DSP**), resistant to race 1; New Era (**NE**), resistant to races 1 and 2; New Season (**NS**), resistant to races 1, 2, and 6; WSU 23 (**23**), resistant to races 1, 2, and 5; and WSU 28 (**28**), resistant to races 1, 5, and 6. **ck** = water-inoculated control. **R1**, **R2**, **R5**, **R6** = races 1, 2, 5, and 6, respectively.

Table 1 lists 26 sites in western Washington in which the current differential varieties did not accurately identify the dominant race or races of wilt in that specific soil. Most of these sites are infested with a mixture of races including the combinations of races 2 plus 5 and 5 plus 6. Survey data are currently being obtained in western Washington to determine if races of *F. oxysporum* f. sp. *pisi* are continuing to spread, if new races are developing, and if these races occur in other growing areas of North America.

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