A Reappraisal of the Race Classification of Fusarium oxysporum f. sp. pisi

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ABSTRACT

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Based on data collected independently at Mt. Vernon and Prosser, Washington, there appeared to be no valid reason for accepting the recently published race classification scheme for *Fusarium oxysporum* f. sp. *pisi* wherein 11 races of the fungus were defined. Our results strongly suggest that the 11-race classification is based more on virulence differences than on true genetic differences in the host, and these same 11 races should be grouped into either race-1 or race-2 types. To minimize the possibility of cultural variants being classified as new races of *F. oxysporum* f. sp. *pisi*, the following criteria are suggested: (i) the isolate must be associated with a prevalent wilt disease under field conditions, (ii) the isolate can be distinguished from other known races of *F. oxysporum* f. sp. *pisi* by a known gene difference in the host.

Wilt of pea, which is caused by *Fusarium oxysporum* Schl. f. sp. *pisi* (van Hall) Snyd. & Hans. race 1, was first described in 1925 (12). Resistance to this disease was quickly found and was attributed to a single, dominant gene factor in the host (17, 18, 19). Race 2 was recognized and described when race-1-resistant cultivars were developed and grown to the exclusion of race-1-susceptible cultivars (15). Like race 1, host resistance to race 2 was quickly found and resistance again was attributed to a separate, dominant gene-factor in the host (7). Delwiche Commando was the first commercial cultivar developed which was resistant to both races 1 and 2. Race 3, described in 1951 (13) in The Netherlands, caused wilt on cultivars resistant to 1 and 2. However, no description of the differential cultivars used to define race 3 and unavailability of an isolate of the original culture makes the validity of this race questionable (8). Race 4, described in 1966 in Canada (2), was distinguished on the basis that cultivar New Era (resistant to race 1 and 2) was susceptible, and cultivar New Wales (resistant to 1 and 2) was resistant. However, it is probable that races 3 and 4 are more virulent cultures of race 2 (8). In addition, the genetic basis for resistance in the host to races 3 and 4 was not defined. In 1970, race 5 was described whereby all cultivars known to be resistant to races 1 and 2 were susceptible (6). Race 5 is a serious economic threat to pea production in northwestern Washington and resistance which was found in U. S. Department of Agriculture Plant Introduction accessions again was attributed to a single dominant gene (5, 10).

Previous research with *F. oxysporum* f. sp. *pisi* has emphasized the importance of optimum soil and air temperatures, nutrient status of the host, and inoculum concentration when testing pea cultivars for wilt resistance (4, 14, 20). Cultural variation within the species *F. oxysporum* is well documented (3, 16) so that preservation of the genotype or "wild type" is essential. This variability has necessitated the use of single-spore isolates with selection to maintain the wild type (i.e. white, restricted, aerial, mycelial colonies for *F. oxysporum* f. sp. *pisi* race 1 and 5 and colonies forming sporodochia for race 2). In addition to single-spore culturing, cultures of *F. oxysporum* f. sp. *pisi* should be maintained in a dormant state (16).

Recently, eleven races of *F. oxysporum* f. sp. *pisi* were described (1), including previously described races 1, 2, 3, 4, and 5. This classification was based on the differential reaction of 27 pea cultivars. Two important considerations concerning this paper are: (i) the techniques and inoculation procedures have not been used by any other pea breeders or pathologists (4, 6, 7, 8, 14, 17, 20) and (ii) races 6-11 [sensu Armstrong and Armstrong (1)] originally were designated as either race 1 or 2 (1). The overriding issue here, we feel, is whether the virulence or uniqueness of races 6-11 are such that they should remain as valid and separate races of *F. oxysporum* f. sp. *pisi*. This paper attempts to answer this question of validity by (i) examining isolates from the American Type Culture Collection listed as race 1-11 with respect to virulence on several differential cultivars at two different locations and (ii) comparing the ATCC isolates

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to isolates of races 1, 2, and 5 routinely used for resistance-breeding tests in our laboratories.

MATERIALS AND METHODS

Acquisition of Fusarium isolates and inoculum production.—Test isolates of *F. oxysporum* f. sp. *pisi*, designated as races 1-11 (1), were purchased from the American Type Culture Collection (ATCC) Rockville, Maryland. These isolates were designated as race 1 (ATCC 26043); race 2 (ATCC 26044); race 4 (ATCC 26087); race 5 (ATCC 16607); race 6 (ATCC 16606); race 7 (ATCC 26045); race 8 (ATCC 26046); race 9 (ATCC 26047); race 10 (ATCC 26048); race 11 (ATCC 26049). For comparison purposes, our isolates were recovered from field-grown plants infected with race 1 (Steptoe, Washington), race 2 (Prosser and Mt. Vernon), and race 5 (Mt. Vernon).

All cultures were derived from single spores on 2% water agar, increased on fresh PDA (16), under artificial light with a 12-hr photoperiod. Only colonies appearing to be representative of the wild type were maintained in soil tubes (2 ml of a conidial suspension placed in 10 gm of a sterile soil mix in a test tube).

To produce inoculum of each test isolate, a small amount of infested soil from the soil tube was sprinkled on a PCNB plate (16) and a resulting colony was selected which appeared representative of the wild type for each isolate. A small agar plug from the colony margin, after a 5-day incubation period, was cut and removed with a No. 6 cork borer, placed in 50 ml of sterile Kerr's medium (9), and incubated for 5 days in a rotary shaker (1 cycle/sec) with 16 hr of fluorescent light at approximately 6,480 lux at 24 ±1 C. At that time, spore concentrations for each isolate were determined by use of a haemocytometer and adjusted to 1×10^6 conidia/ml.

Seed source and planting procedure.—Identities of seed of the various test cultivars and breeding lines used in this study, their source, and reactions to races 1, 2, and 5 of *F. oxysporum* f. sp. *pisi* are listed in Table 1. Seed of each test line was surface disinfested with a 10% solution of Clorox before planting in coarse, autoclaved sand or Perlite.

All seedlings were inoculated in the third- to fourth-node stage by carefully removing each plant, dipping and pruning the root system with a razor blade while the root was immersed in a conidial spore suspension of each isolate. Inoculated seedlings were transplanted back into the planting medium and incubated on a greenhouse bench until wilt symptoms were evident and/or known-susceptible inoculated controls were dead. Greenhouse temperatures were in the 18-24 C range during the duration of this study. Wilt symptoms consisted of stunting, yellowing, dying of lower leaves, downward curling of leaf margins, and usually death of the plant.

TABLE 1. Vascular wilt reaction of test cultivars and breeding lines of peas inoculated with three races of *Fusarium oxysporum* f. sp. *pisi*

Cultivar	Seed source	Wilt reaction		
		Race 1	Race 2	Race 5
Little Marvel	Burpee Seed Co.	S	S	S
W.R. Alaska	Burpee Seed Co.	R	S	S
Dark Skin Perfection	Crites-Moscow Seed Co.	R	S	S
New Era	U. of Wisconsin	R	R	S
New Season	U. of Wisconsin	R	R	S
New Wales	U. of Wisconsin	R	R	S
W.S.U. 23	W. A. Haglund (5)	R	R	R
74SN5	J. M. Kraft (10)	R	R	R

RESULTS

In several repeated pathogenicity tests conducted both at Mt. Vernon and Prosser, Washington, the results did not support the designation of ATCC cultures 26087, 16607, 16606, 26045, 26046, 26047, 26048, and 26049 as races 4-11 of *F. oxysporum* f. sp. *pisi* (Tables 2 and 3). These same isolates could be grouped into either a race-1 type (able to wilt only Little Marvel) or a race-2 type (able to wilt Little Marvel, Wilt-Resistant Alaska, and Dark Skin Perfection but not New Era, New Wales, New Season, W.S.U. 23, or 74SN5. Our race-5 cultures, in comparison to ATCC 16607, induced wilt in cultivars with resistance to races 1 and 2 but not W.S.U. 23 and 74SN5 which are known to be resistant to race 5 (5, 10).

DISCUSSION

The Pacific Northwest is currently a "hot spot" within the pea-growing areas of the world where Fusarium wilt of peas is a serious economic threat (6, 11). This area is a location where new races of *F. oxysporum* f. sp. *pisi* are occurring so that the recent reclassification of the pea wilt pathogen into 11 races is of vital concern to us. The reclassification of *F. oxysporum* f. sp. *pisi* into 11 races (1), according to our data presented in Tables 2 and 3, is based primarily on degrees of virulence.

Using procedures described in this paper, none of the ATCC isolates caused a high incidence of wilt (60-100%) (1) of cultivars with known single-gene resistances to races 1 and 2. We feel that standardized procedures are of extreme importance in determing the true pathogenicity of an isolate of *F. oxysporum* f. sp. *pisi*. Virulence of pathogens is influenced by environment and can be quite variable if inoculum concentration and environment variables are not adequately controlled. For example, we have observed that inoculum concentrations in excess of 10×10^6 microconidia/ml of spore suspension can induce a cortical rot which can be confused with vascular wilt symptoms. Schroeder and Walker (14), and Wells et al. (20) reported that resistant plants appeared to be susceptible if temperatures were high and host-nutrient concentrations were low during incubation.

It is of more than passing interest that races 6-11 [sensu Armstrong and Armstrong (1)] were derived from cultures originally designated as races 1 or 2 (1). There appears to us no conclusive evidence to indicate that races 6-11 of *F. oxysporum* f. sp. *pisi* are distinct from races 1 and 2. In addition, we believe that race 3 (13) and race 4 (2), and that the ATCC culture (16607), listed as race 5, also should be grouped into a race-1 or -2 type.

To minimize the possibility of pathogenic variants with different virulence being classified as new races of *F. oxysporum* f. sp. *pisi*, it is suggested that the following criteria be used: (i) the wilt-type isolate must be associated with a prevalent wilt disease syndrome under field

TABLE 2. Percent vascular wilt of selected pea cultivars and breeding lines inoculated with various isolates and known races of *F. oxysporum* f. sp. *pisi*

Cultivars and breeding lines	Percent vascular wilt caused by isolates of *F. oxysporum* f. sp. *pisi*												
	Race 1 Group					Race 2 Group							
	26043	16607	16606	26045	K-R 1[a]	26044	26087	26046	26047	26048	26049	K-R 2	K-R 5
Little Marvel	50[b]	57	52	10	100	100	91	83	100	62	86	100	100
W.R. Alaska	10	11	30	7	5	78	35	100	88	70	51	96	85
D. S. Perf.	0	23	31	4	3	59	66	79	86	63	25	100	72
New Era	16	13	24	0	17	41	23	12	29	15	14	33	97
New Wales	17	14	28	0	0	14	5	29	24	13	5	9	89
New Season	0	0	0	0	10	30	7	0	35	0	0	10	100
W.S.U. 23	0	17	29	0	0	0	0	0	19	0	20	0	8
74SN5	0	4	0	0	0	25	0	0	5	0	0	0	8

[a]Isolates designated as K-R1, K-R2, and K-R5 were isolated from plants infected with race 1, 2, and 5, respectively, by J. M. Kraft.
[b]Tests were conducted at Prosser, WA and these data are an average of at least three tests of 20 seeds per cultivar.

TABLE 3. Percent vascular wilt of selected pea cultivars and breeding lines to various isolates and designated races of *Fusarium oxysporum* f. sp. *pisi*

Cultivars	Percent vascular wilt caused by isolates of *F. oxysporum* f. sp. *pisi*												
	Race 1 Group			Race 2 Group									
	26043	26045	H-R1[a]	26044	26087	16607	16606	26046	26047	26048	26049	H-R2	H-R5
Little Marvel	53[b]	50	100	98	98	83	60	100	98	95	85	100	95
D. S. Perf.	0	0	16	98	98	80	85	100	95	83	70	98	98
New Era	0	0	13	8	0	40	38	3	8	3	3	3	100
New Wales	0	0	0	20	5	18	35	10	15	5	0	20	98
New Season	0	0	0	6	15	3	0	8	30	15	3	20	98
W.S.U. 23	0	0	6	15	3	20	50	3	15	5	0	3	0

[a]Isolates designated H-R1, H-R2, and H-R5 were isolated by W. G. Haglund from plants infected with race 1, 2, and 5, respectively.
[b]Tests were conducted at Mt. Vernon, WA and these data are an average of five tests of eight seeds per cultivar.

conditions; (ii) the isolate can be distinguished from other known races of *F. oxysporum* f. sp. *pisi* by gene differences in the host; (iii) cultures must be single-spored and stored in a dormant state, preferably in sterile soil tubes; and (iv) wilt inoculations should be conducted under a standardized set of environmental conditions.

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