Another Approach to Race Classification of *Fusarium oxysporum* f. sp. *pisi*

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ABSTRACT

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The validity of the races of *Fusarium oxysporum* f. sp. *pisi* that we differentiated has been challenged. Kraft and Haglund stated that differentiation was due chiefly to low virulence of the isolates and the method of inoculation. They obtained the isolates that we deposited with the American Type Culture Collection (ATCC) and tested them on a limited number of cultivars with their methods. They accepted as valid at that time races 1, 2, and 5, based on isolates from fields in the northwestern USA and the reactions of a few cultivars. We maintain that the small number of isolates and cultivars used by them was too limited to test the validity of all races. It was only after using a large number of cultivars and isolates from widely separated localities that the full potentialities of *F. oxysporum* f. sp. *pisi* became evident. Moreover, they did not completely agree with each other on the race designation of some of our isolates, and some isolates did not show low virulence on certain cultivars in their experiments. We obtained five isolates from the ATCC and simultaneously tested them on key differentials by our method and theirs. No clear evidence of low virulence was found, and we do not understand why they obtained so little wilting in some of their experiments. In our tests, plants were killed quickly with their method, while, with ours, infected plants progressed through the syndrome of disease as seen in the field. Criteria for determining races are discussed.

Additional key words: garden pea.

Kraft and Haglund (14) obtained isolates of the races of *Fusarium oxysporum* Schlecht. f. sp. *pisi* (van Hall) Snyd. & Hans. that we (3) deposited with the American Type Culture Collection (ATCC), Rockville, MD, and concluded, without using our differentials, that they could be grouped into race 1 and race 2. However, they did not agree on the race designation of some of our isolates nor the virulence of some isolates on certain cultivars. Furthermore, they stated that we defined 11 races based on the reaction of 27 cultivars. We (3) identified 10 races and discussed the questionable status of Schreuder's race 3 (17). Twenty-seven pea cultivars (*Pisum sativum* L.) were tested, but only seven cultivars were selected as differentials. They claim that our classification is invalid since it is based on the use of isolates of low virulence, giving as proof the results of their inoculations with the isolates from ATCC, and the use of techniques and inoculation procedures not used by other pea breeders or pathologists.

From experience gained over many years with the wilt fusaria, we (4,5) have suggested some factors that might influence experimental results, as variation in pathogens, temperature, methods of testing, nature and concentration of the inoculum, media for growth of plants, and host-pathogen specialization. We obtained five isolates from the ATCC to simultaneously compare our methods and those of Kraft and Haglund (14). Since we question their interpretation of our data, and since some of the results obtained by Kraft disagree with those obtained by Haglund, an analysis is in order.

MATERIALS AND METHODS

Sources of isolates. Five selected isolates that had been used in the early investigation (3) were obtained from the ATCC; race 1 (ATCC 26043), race 5 (ATCC 16607), race 6 (ATCC 16606), race 7 (ATCC 26045), and race 11 (ATCC 26049). A few isolates maintained in our laboratory also were tested on a few cultivars. A distinction should be made between the isolates of race 5 (ATCC 16607), that was isolated by us from plants in fields in South Carolina, an isolate from the state of Washington deposited by Roberts (ATCC 22554), and an isolate received from Haglund used in the original experiments (3).

Sources of seed. The pea cultivars used in this investigation were WR Alaska 5165 (1978), Burpee Seed Co., Warminster, PA 18974; Dark Skin Perfection, Crites-Moscow Seed Co., Moscow, ID 83843; R702,147, Asgrow Seed Co., Twin Falls, ID 83301; Double One, R. J. Mansholt's, Veredelingsbedrijf. B. V., The Netherlands; Finette (Roi des Fins Verts), B. Kies RIVRO, Wageningen, NL-6140, The Netherlands; Little Marvel, Old's Seed Co., Madison, WI 53706; and WR Little Marvel, Burpee Seed Co.; New Era, Univ. of Wisconsin, Agronomy Dept., Madison, WI 53706, Asgrow Seed Co.; and PI 244195, U.S. Department of Agriculture, Regional Plant Introduction Station, Geneva, NY 14456; New Wales, Univ. of Wisconsin; and Lincoln, Rogers Brothers Seed Company, Twin Falls, ID 83301. Hereafter, seed sources will be indicated by capital letters in parentheses following the cultivar name: Burpee (B) and Old's (O).

Techniques and inoculation procedures. All isolates were used to inoculate cultivar WR Alaska and the susceptible Little Marvel, and, with one exception, WR Little Marvel, Finette, and Double One. Fewer isolates were used on the other cultivars.

Plants were grown during the fall, winter, and spring, usually in sand in 8-L glazed pots (22 cm diameter) steamed at 1.05 kg-force per square centimeter (15 psi) for 8 hr or more and were fertilized with a nutrient solution. Usually 20 seeds were planted per pot, spaced evenly in a circle 2.5 cm from the periphery and 2.5 cm deep. The roots of all plants were cut by pressing an inverted Büchner funnel into the sand. The 3-day-old liquid inoculum, which consisted of mycelial fragments, microspores, and bud cells, was poured around the cut roots. The greenhouse thermostat was set at 28 C in the early work and at 26 C in the present study. The greenhouse was equipped with an automatic top ventilator and pad and fan cooling. Further details are given elsewhere (1–3,5).

RESULTS AND DISCUSSION

Different genomes for resistance in seed lots with the same name. Cultivars of crops other than peas with the same name, but with different genomes for resistance, were encountered in the early stages of our study of the wilt fusaria. We were aware, therefore, that results from the use of commerical cultivars, or even breeding lines, of peas would probably give some inconsistencies in race structures. We (3) showed that different seed lots of New Wales from a single source carried different genomes for resistance. There

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is the distinct probability that plants of New Wales with the same genomes were used by Bolton et al (6) and Kraft and Haglund (14), but these genomes were not the same as those in the plants used by us (3).

Also, it is probable that the Dark Skin Perfection used by Kraft and Haglund was different from ours. Information from P. Matthews (15) (at the John Innes Institute) about Dark Skin Perfection made this fairly conclusive. Discussing his results with flat-bed acrylamide gel electrophoresis in the separation of root-isoperoxidases, he says (*personal communication*, May 1973), "These studies have shown quite conclusively that Dark Skin Perfection exists as two distinct genotypes, i.e., two varieties; in addition certain accessions are undoubtedly mixtures of the two genotypes." The wilt reactions of his Dark Skin Perfection from the USA were not exactly the same as those given by Kraft and Haglund (14).

Even though all the cultivars may not be ideal for differentiating races, their use will be necessary until a sufficient number of pure lines with proven genomes is available. The parameters of the races can be shown only by the wilt reactions of specific cultivars, and it remains to be shown if only cultivars with known single dominant gene resistance are necessary to establish these parameters. It appears that only two of the six differentials given recently (11) (WSU23 and WSU28) are in this class. However, the four races of Haglund and Kraft (11) could not be differentiated by using only these two cultivars.

Methods of inoculation. In the method of Kraft and Haglund (14), plants were grown in coarse autoclaved sand or Perlite to the third- to fourth-node stage, then removed, the roots were cut with a razor blade while immersed in the inoculum suspension, and transplanted back into the planting medium on a greenhouse bench. This method, with some variations, was developed by Hare et al (12) and by Wells et al (20) in a study of the genetic basis of resistance. We found this method so severe that a modified technique of cutting the tap root and a few side roots with scissors about 3 cm below the cotyledons was used so that a comparison of the results with our method (3) could be made. Without using our method, Kraft and Haglund criticize the procedures and generalize that it has not been used by other pathologists working with peas. Our method, which is essentially that of Tharp (18), has been used satisfactorily for many years throughout our investigations of the wilt fusaria and has given symptoms of disease and host responses similar to those encountered in the field. Also, our results with 40 or more other genera and species have compared very well with those of other workers using various methods in the differentiation of susceptible or resistant cultivars and especially in the detection of *formae speciales* and races. The method has been used successfully with eight legumes, viz., silk tree (*Albizzia julibrissin* Durazz.), alfalfa (*Medicago sativa* L.), bean (*Phaseolus vulgaris* L.), *Cassia tora* L., cowpea (*Vigna unguiculata* (L.) Walp.), garden pea, lupine (*Lupinus* spp.) and soybean (*Glycine max* (L.) Merr.) (4,5).

Dixon and Doodson (8) reported on methods of inoculating pea seedlings with Fusarium wilts, in which the best results were obtained by plunging a scalpel into the compost around the plant. They concluded that there was no need to uproot the plants and remove part of the root system. This method of inoculation was similar to our method. However, in our experiments when Little Marvel or New Wales were planted in a pot where a susceptible cultivar had wilted and then grown without any root disturbance, 85% of the plants of Little Marvel wilted with race 1, 70% with race 2, and 92% of New Wales with race 9, which indicated that cutting of roots was not necessary for wilting to occur.

Effect of temperature. Since the optimum soil temperature for the development of wilt caused by races 1 and 2 of *F. oxysporum* f. sp. *pisi* are reported to be somewhat different (19), we were concerned that temperature might be a limiting factor in the greenhouse operated at about 26–28 C. Virgin and Walker (19) stated that wilt (due to race 1) was most rapid at a soil temperature

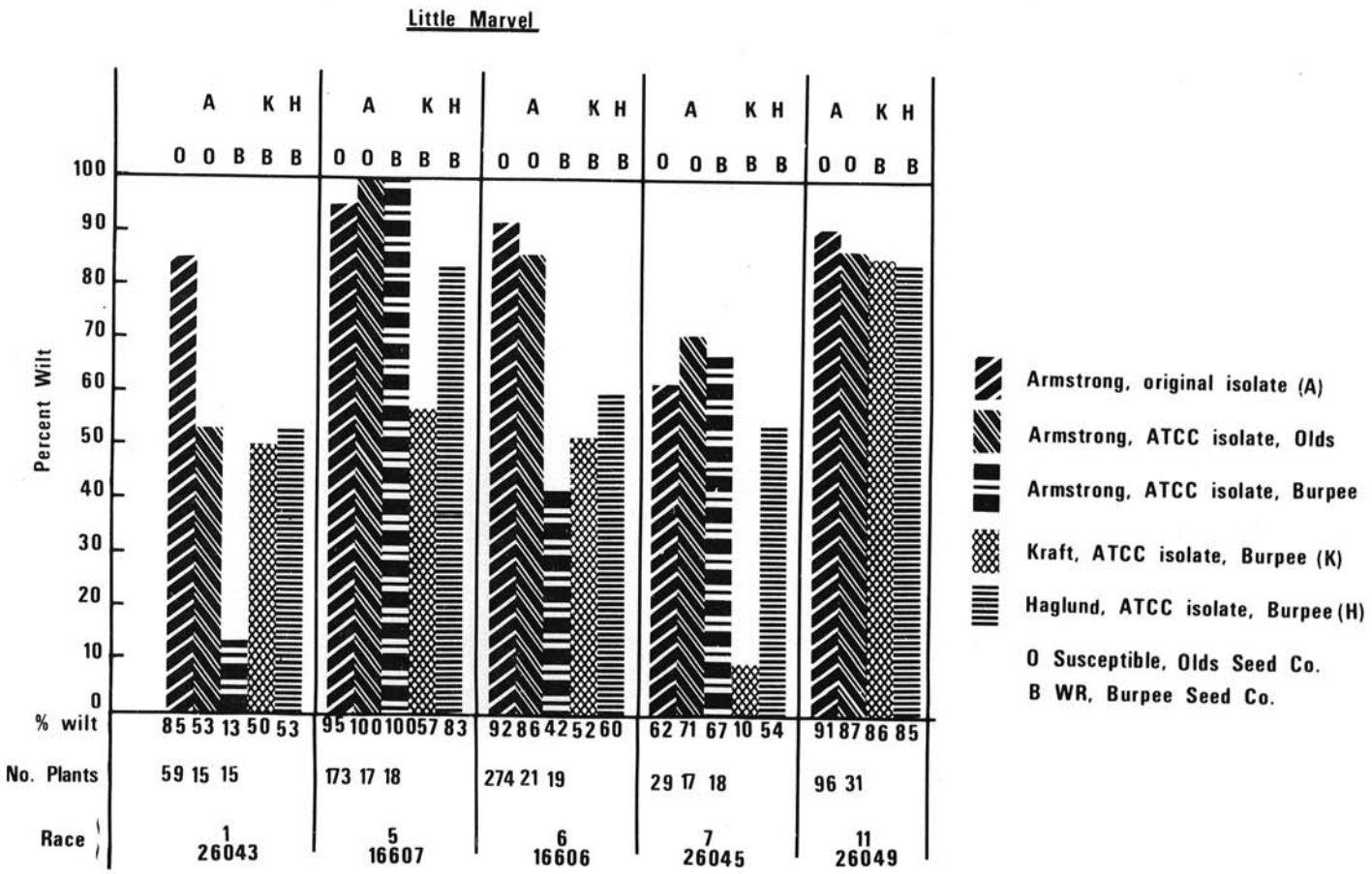

Fig. 1. Comparison of percentages of wilted plants of pea cultivar Little Marvel inoculated with isolates of *Fusarium oxysporum* f. sp. *pisi*. Results of Armstrong with original isolates (3) and Armstrong, Kraft, and Haglund with ATCC isolates.

of 20 C but was nearly as rapid at 24, 28, and 30 C while near-wilt (due to race 2) was most rapid at 24 C but little different at 20 and 28 C. Any differences in host/pathogen interactions that we noted did not appear to be due to temperature as a limiting factor.

Virulence of isolates and designation of races by specific cultivar reaction. The claim of low virulence of the isolates was not evident in the original experiments (3) nor in those with the isolates recently received from ATCC, ie, races 5, 6, 7, and 11 with the susceptible Little Marvel (O) or with races 5 and 7 with WR Little Marvel (B) (Fig. 1). There was an indication of lower virulence of race 1 on Little Marvel (B) in a single-pot test (Fig. 1), but this was not seen with Double One (Fig. 2A) or Finette (Fig. 3). The low percentage of wilt of WR Little Marvel (B) plants inoculated with race 6 indicated either an intermediate degree of resistance in the host or a change in virulence of the isolate. But significantly low virulence was not evident for race 6 or races 5, 7, and 11 in the tests of WR Alaska (Fig. 4) and for different combinations of races in the tests of New Era (Fig. 5), Dark Skin Perfection (Fig. 6), Double One (Fig. 2A), Lincoln (Fig. 2B), and Finette (Fig. 3).

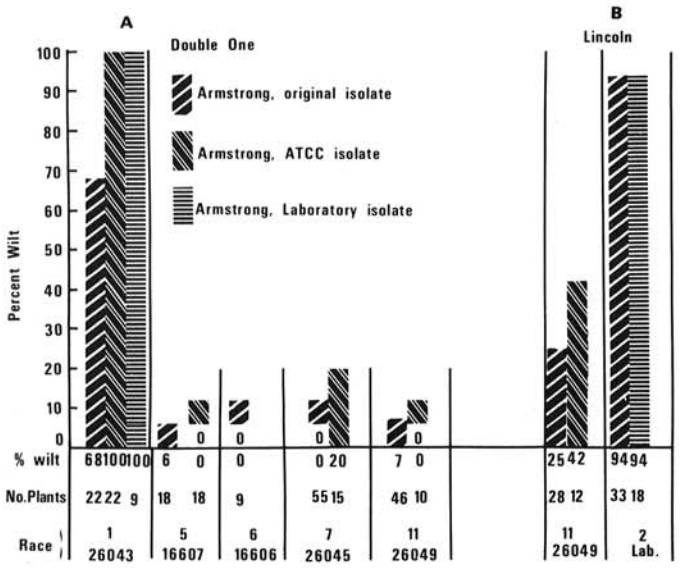

Fig. 2. Comparison of percentages of wilt in pea plants inoculated with *Fusarium oxysporum* f. sp. *pisi*. **A,** Cultivar Double One. Results with original isolates (3), an isolate of race 1 stored in the laboratory, and ATCC isolates. **B,** Cultivar Lincoln. Results with race 11, an original isolate (3), and an ATCC isolate; and race 2 an original isolate (3) and an isolate stored in the laboratory.

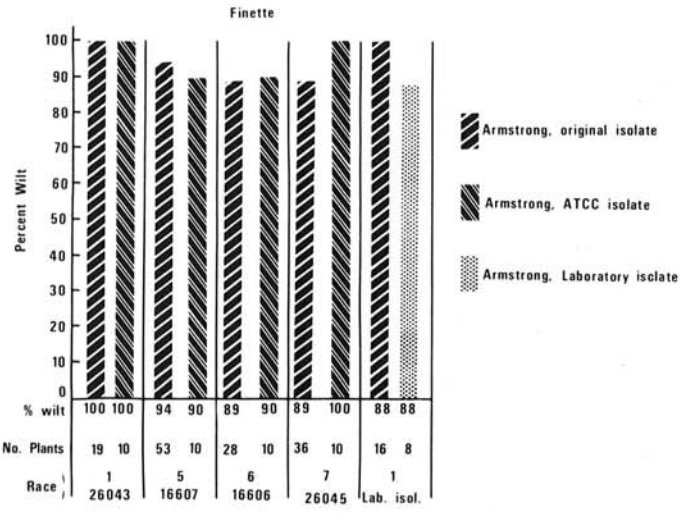

Fig. 3. Comparison of percentages of wilted plants of pea cultivar Finette inoculated with isolates of *Fusarium oxysporum* f. sp. *pisi*. Results with original isolates (3), ATCC isolates, and a race 1 isolate stored in the laboratory.

The plants from various lots of seed of Alaska tested over many years showed wide variations in disease reaction from susceptible to moderately resistant, a condition noted by several early investigators of wilt resistance. In the original study (3) most plants of WR Alaska were killed by races 5, 6, and 11, but in the present investigation complete necrosis did not occur with numerous plants, which suggested a line of Alaska with greater resistance than the original one. The overall symptoms of disease were similar in the two lots, but, in the latter lot, they developed more slowly with our method. However, plants of this lot of WR Alaska that were inoculated with races 5 and 6 by the modified severe technique of cutting roots were dead in 9 days and those inoculated with race 11 in 14 days, but they did not wilt following inoculation with race 7 (Fig. 4). These susceptibility/resistance reactions agreed with those obtained originally (3).

Since Burpee (B) could supply only WR Little Marvel, and Kraft and Haglund obtained seed from that source, we assumed that they were testing the WR line. The five races from ATCC that we tested were among those diagnosed by them as having low virulence. Nevertheless, Kraft (Table 2 in reference 14) reported that 83–100% of the plants of Little Marvel wilted after inoculation with five of the 10 ATCC isolates and 50–62% with four others. In the tests by Haglund (Table 3 in reference 14) 83–100% of the plants wilted with seven of the isolates and 50–60% with the other three. These were isolates claimed to be of low virulence. With Dark Skin Perfection, Haglund (14) obtained wilting of 80–85% of the plants inoculated with races 5 and 6 and placed them in the race 2 group while Kraft (14) obtained 23–31% wilt and placed them in the race 1 group (Fig. 6). These contrasting results are difficult to explain.

In the tests by Haglund (14), 20% of the WSU23 plants wilted after inoculation with race 5, 50% with race 6, but 0% with his highly virulent race 5. Comparable results obtained by Kraft (14) were 17, 29, and 8%, respectively. Thus, the "low virulent" isolates caused the highest percentages of wilt.

The use of their method of inoculation with cultivars New Wales and WR Alaska resulted in more severe reactions than we expected. A modification of the method in which only the tap root was cut with scissors was then tried. However, when plants of New Era were inoculated by this modified technique with isolates of races 5 and 6 and an isolate of each race kept in our laboratory (Fig. 5), almost all plants were dead in 13 days. There were three instances in our early experiments (3) with New Era (66 plants) and two with New Wales (64 plants) in which the plants, growing side by side, were inoculated with either our race 5 or race 5 from Haglund with the percentages of wilted plants among the pots varying from 84–100%. We are puzzled by the low percentages of wilt and lack of race differentiation obtained by Kraft and Haglund with these cultivars

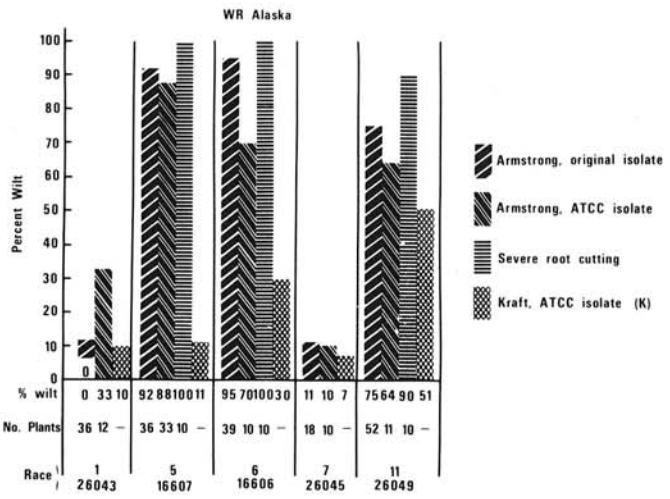

Fig. 4. Comparison of percentages of wilted plants of pea cultivar WR Alaska inoculated with isolates of *Fusarium oxysporum* f. sp. *pisi*. Results of Armstrong with original isolates (3) and Armstrong and Kraft with ATCC isolates.

and isolates.

They (14) stated that our races 6–11 were designated originally as races 1 and 2, and there appeared to be no conclusive evidence in their experiments to indicate that they were distinct from races 1 and 2. However, they did not use all our differentials when screening the ATCC isolates; consequently, some of the races that we found would not be evident. Thus, if the two cultivars, Little Marvel and Double One, were chosen, only races 1 and 2 would be evident (Table 1).

Race 6 was received as a Wisconsin race 1. Kraft (14) placed it in race 1, and Haglund (14) placed it in race 2. The reaction of a number of cultivars that we (3) tested suggested that it might be race 1, but this was not true since the susceptible (S) reaction of four cultivars, including WR Alaska (Fig. 4) and New Era from the three sources (Fig. 5), should have been resistant (R) if race 1 and the R reaction of Double One should have been S (Fig. 2A).

Likewise, we (3) have shown by specific cultivar reactions that race 7 (Fig. 2A) and race 11 (Figs. 2A and 4), received as race 1, were not race 1. Furthermore, race 5 collected in South Carolina was not race 1 (Figs. 2A, 4–6) as given by Kraft (14) or race 2 (Figs. 5 and 6) as given by Haglund (14) but was race 5 as described by them (10).

The isolates of races 5 and 6 were sent to the ATCC in April 1966, lyophilized and frozen under nitrogen, and returned for checking in August 1966. No change in virulence was noted. They were returned again in December 1978 and tested on New Era along with isolates that had been routinely grown on potato-dextose agar and stored at 5 C (Fig. 2). No changes in the virulences of these isolates were detected after 12 yr. However, race 5 that was sent to the ATCC (22554) by Roberts was avirulent on New Era and Finette and was not tested further.

Criteria for the differentiation of races. Some criteria for the differentiation of races of *F. oxysporum* f. sp. *pisi* as stated by Haglund (9) are, "the isolate must occur in nature," "the isolate must become established in nature and survive with time," "the isolate must be of economic importance." Kraft and Haglund (14) stated that to be valid for establishing a race, "the isolate can be distinguished from other known races of *F. oxysporum* f. sp. *pisi* by gene differences in the host." We (3) showed that two lots of seed of New Wales from one source had different genomes for resistance; however, neither lot had proven single dominant gene resistance. Probably the same was true for two lots of Dark Skin Perfection. Which lot should have been accepted? Are all races of other *formae speciales* of *F. oxysporum* causing wilt invalid because the genetics of resistance is unknown? With present methods, the use of resistant and susceptible cultivars now available has been and probably will continue to be useful in the differentiation of races. They (14) also stated that "cultures must be single-spored and stored in a dormant state, preferably in sterile soil tubes." General methods of storing cultures were mentioned by Jong and Davis (13). The most serious objection to the soil tube method was given by Booth (7). Mutation in sterile soil culture has been reported for f. sp. *melonis* (16). We (3–5) have suggested that cultures should be deposited at central locations such as the ATCC for long-term preservation by freeze-drying (lyophilization) and cryogenic storage in liquid nitrogen. They (14) stated further that "the wilt-type isolate must be associated with a prevalent wilt disease syndrome under field conditions." Was it probable that the cultures (3) from Doling and Taylor in England, Lawyer and Reiling in the United States, Bolton in Canada, Kerr in Australia, and Hubbeling in The Netherlands did not fit these criteria or that all became laboratory mutants in the relatively short time before they were used?

Haglund and Kraft (10) in 1970, accepted as valid, races 1–5. In Table 1 (10), they listed Rondo, Wisconsin Perfection, and Delwiche Commando as differentials for race 3, "reaction based on published data" of Schreuder (17), but there is no mention of these cultivars in that paper. Our analysis of the questionable status of race 3 has been given in detail (3). They also recognized race 4 at that time but in 1978, claimed validity for only races 1, 2, and 5, that had been collected in the Pacific Northwest and differentiated by using a limited number of cultivars and breeding lines developed by them (14). In 1979, they (11) reported race 6 that was capable of wilting pea cultivars resistant to races 1, 2, and 5. If new races are appearing in their fields, why can't other races of f. sp. *pisi* with different genotypes appear elsewhere? With a worldwide crop such

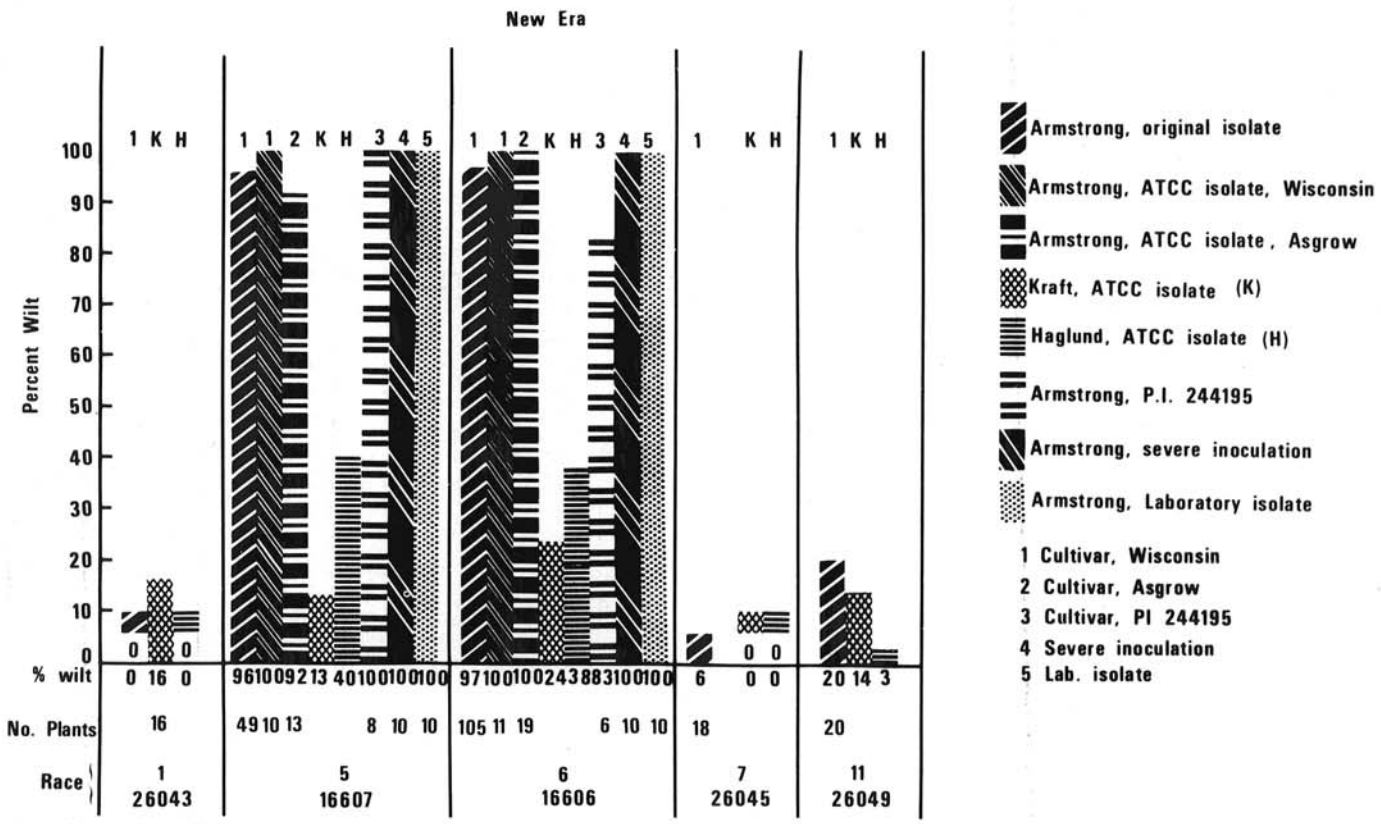

Fig. 5. Comparison of percentages of wilted plants of pea cultivar New Era inoculated with isolates of *Fusarium oxysporum* f. sp. *pisi*. Results of Armstrong with original isolates (3) and Armstrong, Kraft, and Haglund with ATCC isolates.

TABLE 1. Reactions of cultivars of pea to isolates of *Fusarium oxysporum* f. sp. *pisi*

Cultivar	Host response (external symptoms)[a] to races				
	1(26043)	2(26044)	4(26087)	5(16607)	6–11 (inclusive)
Little Marvel	S_9	S_{13}	S_{13}	S_{15}	S_{38}
Double One	S_8	$R_3\ I_3$	R_3	R_4	R_{11}

[a]Symptoms: R = resistant, 0–39% wilt; I = intermediate, 40–59% wilt; S = susceptible, 60–100% wilt. Subscripts = number of tests.

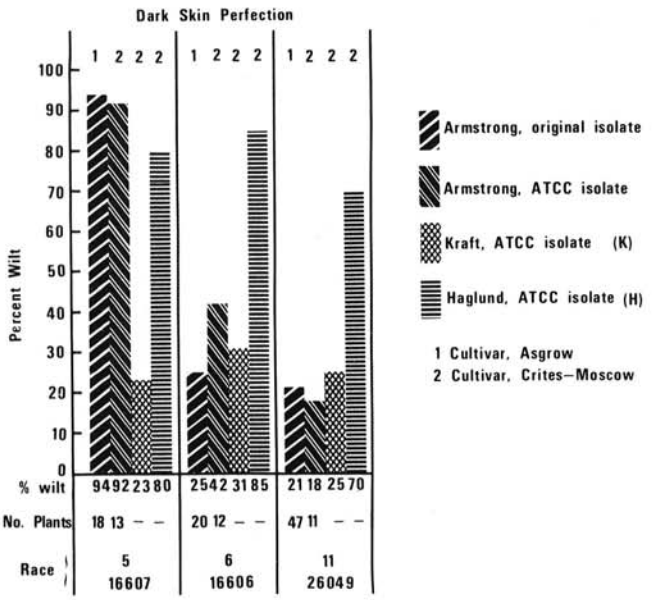

Fig. 6. Comparison of percentages of wilted plants of pea cultivar Dark Skin Perfection inoculated with isolates of *Fusarium oxysporum* f. sp. *pisi*. Results of Armstrong with original isolates (3) and Armstrong, Kraft, and Haglund with ATCC isolates.

as pea cultivated extensively for many years, the appearance of new races of the fungus is not surprising. Numerous races have been discovered for *F. oxysporum* ff. sp. *lini, vasinfectum, melonis,* and two or more races for nine other *formae speciales.*

To attain a measure of standardization, perhaps procedures are now in order similar to those of the investigators of the cereal rusts (not reviewed) and recently by the investigators of club root (also not reviewed) in which a tabulation of methods of each investigator was made. The concepts and procedures that seem to be the most satisfactory for f. sp. *pisi* might then be decided, perhaps at a future conference of the Fusarium research workers.

LITERATURE CITED

1. ARMSTRONG, G. M. 1941. A solution-culture method used in the study of Fusarium wilts. Phytopathology 31:549-553.
2. ARMSTRONG, G. M., and J. K. ARMSTRONG. 1948. Nonsusceptible hosts as carriers of wilt fusaria. Phytopathology 38:808-826.
3. ARMSTRONG, G. M., and J. K. ARMSTRONG. 1974. Races of *Fusarium oxysporum* f. sp. *pisi,* causal agents of pea wilt. Phytopathology 64:849-857.
4. ARMSTRONG, G. M., and J. K. ARMSTRONG. 1975. Reflections on the wilt fusaria. Annu. Rev. Phytopathol. 13:95-103.
5. ARMSTRONG, G. M., and J. K. ARMSTRONG. 1978. Formae speciales and races of *Fusarium oxysporum* causing wilts of the Cucurbitaceae. Phytopathology 68:19-28.
6. BOLTON, A. T., V. W. NUTTALL, and L. H. LYALL. 1966. A new race of *Fusarium oxysporum* f. *pisi.* Can. J. Plant Sci. 46:343-347.
7. BOOTH, C. 1971. The genus Fusarium. Commonw. Mycol. Inst., Kew, Surrey, England. 237 pp.
8. DIXON, G. R., and J. K. DOODSON. 1970. Methods of inoculating pea seedlings with Fusarium wilt. J. Nat. Inst. Agric. Bot. 12:130-135.
9. HAGLUND, W. A. 1974. Race concept in *Fusarium oxysporum* f. *pisi.* Pisum Newsletter 6:20-21.
10. HAGLUND, W. A., and J. M. KRAFT. 1970. *Fusarium oxysporum* f. *pisi* race 5. Phytopathology 60:1861-1862.
11. HAGLUND, W. A., and J. M. KRAFT. 1979. *Fusarium oxysporum* f. sp. *pisi*, Race 6: Occurrence and distribution. Phytopathology 69:818-820.
12. HARE, W. W., J. C. WALKER, and E. H. DELWICHE. 1949. Inheritance of a gene for near-wilt resistance in the garden pea. J. Agric. Res. 78:239-250.
13. JONG, S. C., and E. E. DAVIS. 1978. Conservation of reference strains of *Fusarium* in pure culture. Mycopathologia 66:153-159.
14. KRAFT, J. M., and W. A. HAGLUND. 1978. A reappraisal of the race classification of *Fusarium oxysporum* f. sp. *pisi.* Phytopathology 68:273-275.
15. MATTHEWS, P., and H. WILLIAMS. 1973. Genetics of root isoperoxidase variants in *Pisum.* Rep. John Innes Inst. for 1972 (1973):43-45.
16. McKEEN, C. D., and R. N. WENSLEY. 1962. Cultural and pathogenic variation in the muskmelon wilt fungus. Can. J. Microbiol. 8:769-784.
17. SCHREUDER, J. C. 1951. Een onderzoek over de Americaanse vaatziekte van de erwten in Nederland. Tijdschr. Plantenziekten 57:175-206. (English summary)
18. THARP, W. H. 1938. A sand-nutrient infection technique for the study of Fusarium wilt of cotton. Phytopathology 28:206-209.
19. VIRGIN, W. J., and J. C. WALKER. 1939. Relation of temperature and moisture to near-wilt of pea. J. Agric. Res. 59:591-600.
20. WELLS, D. G., W. W. HARE, and J. C. WALKER. 1949. Evaluation of resistance and susceptibility in garden peas to near-wilt in the greenhouse. Phytopathology 39:771-779.