

**Use of Morphology and Mating Populations
in the Identification of Formae Speciales in *Fusarium solani***

Takken Matuo and William C. Snyder

Professor, Faculty of Textile Science, Shinshu University, Ueda, Nagano-ken, Japan; and Professor Emeritus, Department of Plant Pathology, University of California, Berkeley 94720.

Supported in part by the United States-Japan Cooperative Research Program, "Systematic and Ecological Investigations on *Fusaria* in the Pacific Region".

The authors are pleased to acknowledge the assistance of Dr. Shirley N. Smith in the preparation of the manuscript.

Accepted for publication 13 November 1972.

ABSTRACT

Nine formae speciales and two races of *Fusarium solani* have been reported in the United States and Japan. They are sorted into four groups (A, B, C, D) by the morphologic characters of macroconidia which were formed on sporodochia under continuous illumination. Group A, composed of f. sp. *cucurbitae* race 1 and some isolates of race 2, f. sp. *batatas*, f. sp. *mori*, f. sp. *xanthoxyli*, f. sp. *robiniae* and an isolate of f. sp. *eumartii*, is characterized by predominant 5- (or more) septate macroconidia. The B group, which includes f. sp. *pisi*, f. sp. *cucurbitae* race 2 and several isolates of f. sp. *eumartii*, have predominant 3-septate macroconidia less than 5 μ in width. The C group, f. sp. *radicicola*, is characterized by predominant 3-septate macroconidia which are 5.5 μ or more in width. The D group, f. sp. *phaseoli*, has

predominant 4-septate macroconidia. There was no significant difference in the morphologic characters of microconidia and *Hypomyces* stage, if present, among these formae speciales and races.

Six formae speciales and two races have been known to have a *Hypomyces* stage. In mating and sexual behavior as well as morphology, these formae speciales and races are very similar to *Fusarium (Hypomyces) solani* f. sp. *cucurbitae* race 1, but they are distinctly isolated from one another with regard to mating population. From this fact, it was proposed that the crossing tests will be usable as a method for identifying formae speciales and races, where matings may be made, in addition to the pathogenicity tests.

Phytopathology 63:562-565

Five formae speciales of *Fusarium solani*, namely f. sp. *cucurbitae*, f. sp. *eumartii*, f. sp. *phaseoli*, f. sp. *pisi*, and f. sp. *radicicola* were first named by Snyder & Hansen (15) in 1941. McClure (7) and Toussoun & Snyder (19) added f. sp. *batatas* and f. sp. *cucurbitae* race 2 as a new forma specialis and a new race, respectively, in the United States. Matuo & Sakurai (3, 4) reported the presence of f. sp. *phaseoli*, f. sp. *pisi*, and f. sp. *radicicola* in Japan, and designated in addition the three new formae speciales, f. sp. *mori* (9, 10), f. sp. *xanthoxyli* (13), and f. sp. *robiniae* (5) there. These formae speciales and races in the United States and Japan are summarized in Table 1. A summary of perfect stages in *Fusarium* was reported in 1965 by Snyder & Toussoun (18).

The differentiation of these formae speciales and races depends on selective pathogenicities to their hosts, since they are closely similar in respect to the morphology of the macroconidia, microconidia and *Hypomyces* stage. In comparative studies of the Fusaria of the United States and Japan from 1967 to 1970, particular attention was given both to the morphologic characters and to mating populations, as well as to the pathogenicities of numerous isolates of all the formae speciales and races of *F. solani* from both laboratories. The ability to cross was tested as an aid for identification of formae speciales and races in addition to the pathogenicity tests.

MORPHOLOGIC CHARACTERS OF SPORES.—*Macroconidia.*—Morphologic characters of macroconidia of *Fusarium* vary with the conditions of culture, yet they are almost constant under certain conditions of culture. Sakurai & Matuo (12) investigated the morphologic characteristics of six formae speciales and two races of *F. solani*. Included were two American formae speciales, f. sp. *cucurbitae* and f. sp. *pisi*, which they had received from Snyder. In this investigation, the Fusaria were cultured in potato-sucrose agar (PSA—1% sucrose was added to potato-decoction agar) at 25 C in diffuse daylight, supplemented by 12 hr of fluorescent light. The

formae speciales and races in these tests were sorted into four groups (A, B, C, D) by the morphologic characters of the macroconidia. The A group, f. sp. *cucurbitae*, f. sp. *mori*, and f. sp. *xanthoxyli*, show a characteristic in which 5- (or more) septate macroconidia are predominant (Fig. 1). The B group, f. sp. *pisi* and f. sp. *radicicola* race 2, is characterized by predominant 3-septate macroconidia, less than 5 μ in width, whereas the C type, f. sp. *radicicola* race 1 is characterized by predominant 3-septate macroconidia about 5.5 μ or more in width. The D type, f. sp. *phaseoli*, has predominant 4-septate macroconidia and no microconidia under the conditions of these studies. In the cooperative program between the United States and Japan, we investigated the same morphologic characters of numerous isolates of previously investigated formae speciales and races, and the remaining formae speciales and races, f. sp. *eumartii*, f. sp. *batatas*, f. sp. *cucurbitae* race 2 and f. sp. *robiniae*. Isolates of previously investigated formae speciales and races showed the same results grown under these conditions, and both isolates of f. sp. *batatas*, all 29 isolates of f. sp. *robiniae*, and one isolate of f. sp. *eumartii* proved to belong to the A group. Five isolates of f. sp. *eumartii* and both isolates of f. sp. *cucurbitae* race 2 proved to belong to the B group.

Microconidia and the Hypomyces stage.—Microconidia of *F. solani* are formed on long, septate and occasionally branched conidiophores as Sherbakoff (14) pointed out. The microconidia and conidiophores of these formae speciales and races in the United States and Japan are morphologically alike except for occasional large kidney-shaped microconidia of f. sp. *cucurbitae* race 2, which seem to be unimportant. These similarities preclude any separation. Japanese isolate (12) of f. sp. *phaseoli* has not produced microconidia, but United States isolates of the same forma specialis have occasionally produced them. *Hypomyces* stage (perithecium, ascus, ascospore) is easily formed on potato-dextrose

TABLE 1. Formae speciales and races of *Fusarium solani* in the United States and Japan

Forma specialis and race	Host (symptom type)	Year named	Occurrence
f. sp. <i>cucurbitae</i> Snyder et Hansen. race 1	cucurbitis (foot rot, fruit rot)	1941, 1961	in U.S. —
f. sp. <i>eumartii</i> (Carp.) Snyder et Hansen.	potato (foot rot)	1941	in U.S.
f. sp. <i>phaseoli</i> (Burk.) Snyder et Hansen.	bean (foot rot)	1941	in U.S. & Japan
f. sp. <i>pisi</i> (Jones) Snyder et Hansen.	pea (foot rot)	1941	in U.S. & Japan
	mulberry tree (stem blight)		—
	chick pea (root rot)		—
	ginseng (root rot)		—
f. sp. <i>radicicola</i> (Wr.) Snyder et Hansen.	potato (tuber rot)	1941	in U.S. & Japan
	<i>Amorphophalus</i> (corn rot)		—
	tulip (bulb rot) etc.		—
f. sp. <i>batatas</i> McClure	sweet potato (foot rot)	1951	in U.S.
f. sp. <i>mori</i> Sakurai et Matuo	mulberry tree (stem blight)	1959	in Japan
f. sp. <i>cucurbitae</i> Snyder et Hansen. race 2	cucurbits (fruit rot)	1961	in U.S.
f. sp. <i>xanthoxyli</i> Sakurai et Matuo	Japanese pepper (trunk blight)	1961	in Japan
f. sp. <i>robiniae</i> Matuo et Sakurai	<i>Robinia pseudoacacia</i> (twig blight)	1965	in Japan

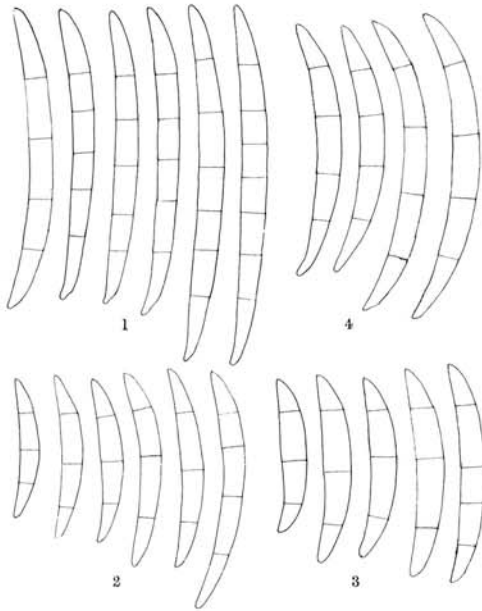


Fig. 1. Four types of macroconidia of *Fusarium solani*. 1) A type; 2) B type; 3) C type; 4) D type.

agar (PDA) or PSA by crossing of opposite mating type clones of two pairs of each f. sp. *cucurbitae* race 1, f. sp. *pisi*, f. sp. *batatas*, f. sp. *mori*, f. sp. *cucurbitae* race 2, f. sp. *xanthoxyli*, and f. sp. *robiniae*. There was no difference in the shape and size of perithecia, asci and ascospores among these formae speciales and races.

MATING POPULATIONS.—Dimock (1) was the first to report the heterothallism of *F. solani*. He studied the phenomenon with *Hypomyces ipomoeae*, a synonym of *F. (Hypomyces) solani* f. sp. *batatas* [McClure (7)]. Hansen & Snyder (2, 16) studied more precisely the heterothallism and the genetics of

F. (Hypomyces) solani with *F. (Hypomyces) solani* f. sp. *cucurbitae* race 1. This fungus was hermaphroditic, had mating (compatibility) types (+), (-), and through mutation developed heterothallism in respect to sex. Hermaphroditic, male and female, and neuter clones of the fungus were found. Sakurai & Matuo (11, 13), Toussoun & Snyder (19), Reichle et al. (8), and Matuo & Sakurai (5) studied the heterothallism of various formae speciales of *F. (Hypomyces) solani*: f. sp. *mori*, f. sp. *xanthoxyli*, f. sp. *cucurbitae* race 2, f. sp. *pisi*, and f. sp. *robiniae*. The results proved that all these formae speciales are very similar to *F. (Hypomyces) solani* f. sp. *cucurbitae* race 1 in mating and sexual behavior (2, 16).

Sakurai & Matuo (12) crossed six formae speciales and two races of *F. solani*: f. sp. *cucurbitae*, f. sp. *mori*, f. sp. *xanthoxyli*, f. sp. *pisi*, f. sp. *phaseoli*, f. sp. *radicicola* race 1, and f. sp. *radicicola* race 2, reciprocally by the spermatizing method. The experimental results proved that the interformic and interracial crosses do not occur except for f. sp. *pisi* × f. sp. *radicicola* race 2. From this fact, *F. solani* f. sp. *radicicola* race 2, one of the causal Fusaria of mulberry stem blight, was identified later with f. sp. *pisi* (4, 8). Toussoun & Snyder (19) reported that *F. solani* f. sp. *cucurbitae* race 2 does not cross with race 1 of the same forma specialis. Matuo & Sakurai (5) reported that *F. solani* f. sp. *robiniae* is independent from four formae speciales of *F. solani* in the mating relationship.

In the cooperative program between the United States and Japan, we endeavored to make clear the mating populations of all formae speciales and races of *F. (Hypomyces) solani* in the United States and Japan, paying special attention to the non-investigated combinations. All isolates were maintained by single spore culture in PDA tubes at room temperature or 25 C in diffuse daylight, or in daylight supplemented by 12 hr of fluorescent light. Crosses were made 20-30 days after single sporing by

TABLE 2. Differentiation of pathogenicity, morphology, and mating population of *Fusarium solani* in the United States and Japan

Forma specialis	Morphologic type of macroconidia ^a	Microconidia ^b	<i>Hypomyces</i> stage ^b	Mating population ^c
f. sp. <i>cucurbitae</i> race 1	A	+	+	I
f. sp. <i>batatas</i>	A	+	+	II
f. sp. <i>mori</i>	A	+	+	III
f. sp. <i>xanthoxyli</i>	A	+	+	IV
f. sp. <i>robiniae</i>	A	+	+	VII
f. sp. <i>eumartii</i>	A	+	-	-
f. sp. <i>pisi</i>	B	+	+	VI
f. sp. <i>cucurbitae</i> race 2	B	+	+	V
f. sp. <i>eumartii</i>	B	+	-	-
f. sp. <i>radicicola</i>	C	+	-	-
f. sp. <i>phaseoli</i>	D	±	-	-

^a A: 5-(or more) septate macroconidia predominant. B: 3-septate macroconidia (less than 5 μ in width) predominant. C: 3-septate macroconidia (about 5.5 μ or more in width) predominant. D: 4-septate macroconidia predominant.

^b Microconidia and *Hypomyces* stage of each forma specialis or race closely resemble one another when present.

^c Roman numerals (I-VII) show independent mating populations which were discovered chronologically.

pouring a suspension of conidia in sterile water from one clone into tubes containing another, and the cultures were kept as they were for 6 months. The results proved that all the formae speciales and races of *F. (Hypomyces) solani* are distinctly isolated from one another with regard to mating population; that is, each of them constitutes the individual mating population as shown in Table 2.

DISCUSSION.—Morphologic characters and mating populations of all the formae speciales and races of *F. (Hypomyces) solani* in the United States and Japan are summarized in Table 2. They were sorted into four groups (A, B, C, D) by the morphologic characters of macroconidia which were formed on sporodochia, though the separation by microconidia and *Hypomyces* stage was difficult. The existence of four macroconidium groups (A, B, C, D) does not mean that the differences are great enough to be used for separation at the species level. Rather, that they represent an additional guide in the sub-specific characterization of formae speciales and races in *F. solani*. Most of the formae speciales or races of *F. solani* belong to a single group, but *F. solani* f. sp. *eumartii* belong to two groups, A and B; thus, such morphology by itself is undependable in classification. This phenomenon is similar to that in *F. roseum* f. sp. *cerealis* which has three cultivars: 'Graminearum', 'Culmorum', and 'Avenaceum' (17).

Though the differentiation of formae speciales and races depends only on their selective pathogenicities to the hosts, they all proved to be distinctly isolated from each other with regard to sexual reproduction. Each of them constitutes an individual mating population. In this respect, *F. (Hypomyces) solani* can be regarded as a unique contrast to other pathogenic fungi: smuts, rust, etc., in which not only inter-racial or intervarietal crosses but also interspecific (intergeneric) crosses were reported. Mating populations have been used in other areas of biology to delimit species. However, use of the mating type in the speciation of the *Fusaria* would deprive the worker of a rapid and useful taxonomic system.

On the other hand, if we have tester clones of known formae speciales, the crossing with them will supply an effective method for identifying formae speciales of *F. (Hypomyces) solani* in addition to the pathogenicity tests. Actually, we (6) identified the clones of *F. solani* isolated from mulberry stems and ginseng roots with *F. solani* f. sp. *pisi* by the crossing tests with the tester clones. Standardized techniques for determining forma specialis and race in *Fusarium* have not been decided upon. Hereafter, standardized techniques may be necessary if we are to have agreement internationally among workers. The crossing tests will surely be available for this purpose in *F. solani*, where matings may be made in addition to the inoculation tests.

As already stated, all formae speciales and races of *F. (Hypomyces) solani* in the United States and Japan having a *Hypomyces* stage were heterothallic. However, some clones of homothallic *F. solani* have

been collected in the United States and Japan, all of which are saprophytic.

LITERATURE CITED

1. DIMOCK, A. W. 1937. Observations on sexual relations in *Hypomyces ipomoeae*. *Mycologia* 29:116-127.
2. HANSEN, H. N., & W. C. SNYDER. 1943. The dual phenomenon and sex in *Hypomyces solani* f. *cucurbitae*. *Amer. J. Bot.* 30:419-422.
3. MATUO, T. 1961. On the classification of Japanese *Fusaria*. *Ann. Phytopathol. Soc. Japan* 26:43-47.
4. MATUO, T., & Y. SAKURAI. 1963. On the relation between *Fusarium solani* f. sp. *radicicola* race 2 and *F. solani* f. sp. *pisi*. *Ann. Phytopathol. Soc. Japan* 28:311 (Abstr.).
5. MATUO, T., & Y. SAKURAI. 1965. *Fusarium solani* f. *robiniae* n. f., one of the causal *Fusaria* of the twig blight of *Robinia pseudoacacia*. *Ann. Phytopathol. Soc. Japan* 30:31-36.
6. MATUO, T., & W. C. SNYDER. 1972. Host virulence and the *Hypomyces* stage of *Fusarium solani* f. sp. *pisi*. *Phytopathology* 62:731-735.
7. MC CLURE, T. T. 1951. *Fusarium* foot rot of sweet-potato sprouts. *Phytopathology* 41:72-77.
8. REICHLER, R. E., W. C. SNYDER, & T. MATUO. 1964. *Hypomyces* stage of *Fusarium solani* f. *pisi*. *Nature* 203:664-665.
9. SAKURAI, Y., & T. MATUO. 1957. On a *Fusarium* disease of mulberry twigs caused by *Hypomyces solani* (Rke. et Berth.) Snyder et Hans. *Res. Rep. Fac. Textile & Sericulture, Shinshu Univ.* 7:18-24.
10. SAKURAI, Y., & T. MATUO. 1959. On the form name and race of *Hypomyces solani* (Rke. et Berth.) Snyder et Hans. which is pathogenic to the mulberry trees. *Ann. Phytopathol. Soc. Japan* 24:219-223.
11. SAKURAI, Y., & T. MATUO. 1959. Heterothallism in *Hypomyces solani* f. *mori*. *Res. Rep. Fac. Textile & Sericulture, Shinshu Univ.* 9:22-27.
12. SAKURAI, Y., & T. MATUO. 1960. Studies on the intraspecific groups in *Fusarium solani*. I. On mating populations and morphologic groups in the species. *Res. Rep. Fac. Textile & Sericulture, Shinshu Univ.* 10:21-32.
13. SAKURAI, Y., & T. MATUO. 1961. Taxonomy of the causal fungus of trunk-blight of *Xanthoxylum piperitum* and heterothallism in this fungus. *Ann. Phytopathol. Soc. Japan* 26:112-117.
14. SHERBAKOFF, C. D. 1953. *Fusaria* associated with citrus feeder roots in Florida. *Phytopathology* 43:395-397.
15. SNYDER, W. C., & H. N. HANSEN. 1941. The species concept in *Fusarium* with reference to section *Martiella*. *Amer. J. Bot.* 28:738-742.
16. SNYDER, W. C., & H. N. HANSEN. 1954. Species concept, genetics, and pathogenicity in *Hypomyces solani*. *Phytopathology* 44:338-342.
17. SNYDER, W. C., H. N. HANSEN, & J. W. OSWALD. 1957. Cultivars of the fungus, *Fusarium*. *J. Madras Univ.* 27:185-192.
18. SNYDER, W. C., & T. A. TOUSSOUN. 1965. Current status of taxonomy in *Fusarium* species and their perfect stage. *Phytopathology* 55:833-837.
19. TOUSSOUN, T. A., & W. C. SNYDER. 1961. The pathogenicity, distribution and control of two races of *Fusarium (Hypomyces) solani* f. *cucurbitae*. *Phytopathology* 51:17-22.