Host Virulence and the Hypomyces Stage of Fusarium solani f. sp. pisi

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ABSTRACT

Fusarium solani f. sp. *pisi*, a widespread soil-borne pathogen, has been shown identical by mating tests to the pathogens causing foot and root rot of pea, branch blight of mulberry trees, and root rot of ginseng. This fungus is heterothallic in mating type and hermaphroditic or unisexual regarding sex. In nature the *Hypomyces* perithecia have been found only on diseased mulberry branches. The inference was thus made that origins of this pathogen and possible sources of new races may be in mulberry tree-growing regions, which include Japan.

Most pea and mulberry tree isolates showed high virulence to pea seedlings, but ginseng isolates showed weak or no virulence to the same pea cultivars. However, both pea and ginseng isolates were virulent to mulberry branches.

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Fusarium solani f. sp. *pisi* occurs in the USA, England, Japan, Australia, New Zealand, and probably elsewhere as the cause of a cortical foot and root disease of pea (1, 10). In addition, it causes a branch blight of mulberry trees in Japan (7, 11, 12) and is credited with inducing a root rot of chickpea

(*Cicer arietinum*) in the USA (5).

The root rot of pea caused by *F. solani* f. sp. *pisi* is important and widespread throughout the pea-growing areas of the USA (16), but is not so prevalent in Japan. The only conspicuous outbreaks there have occurred in the Hokkaido district (4). The fungus, however, has been collected from many districts of Japan as the incitant of branch blight of mulberry trees. It is probable that the small scale of pea cultivation in Japan limits the extent of the fungus on that crop, rather than a lack of pathogen distribution, for Matuo was able to collect isolates from roots of stunted peas cultivated as a garden vegetable in the Nagano Prefecture.

Branch blight of mulberry trees caused by *Fusarium solani* was discovered by Sakurai & Matuo (11, 12) in the Nagano Prefecture of Japan in 1957. The disease has since been found in the Iwate, Gumma, Saitama, Miyazaki, and Kumamoto Prefectures, which suggests its widespread distribution in Japan. It is fairly destructive, though not as common as the disease caused by *Fusarium lateritium* f. sp. *mori* (6, 9). *Fusarium solani* causing branch blight of mulberry consists of two distinct formae speciales: f. sp. *mori* and f. sp. *pisi* (7, 10). The latter, first called *F. solani* f. sp. *radicicola* race 2 (12), was later identified as *F. solani* f. sp. *pisi* because it crossed with California isolates from pea and produced viable ascospores (7, 10). Both *F. solani* f. sp. *mori* and *F. solani* f. sp. *pisi* have their own respective mating populations, and do not cross with each other. The symptoms caused by these two fungi are similar, and the fungi cannot be distinguished except by macroconidia, which are formed on similar sporodochia on mulberry branch lesions (f. sp. *mori*–5-septated dominant, f. sp. *pisi*–3-septated dominant). These sporodochia are the most conspicuous signs of the disease (Fig. 1-C, D). They form most readily at the lower end of the branch, breaking through the cork layer during wet conditions. They are 0.5 to 3 mm in diameter and generally white, light cream-colored, or sometimes cobalt blue, but are distinctly different from the salmon- to brown-colored sporodochia of *F. lateritium* f. sp. *mori*. Typical *Hypomyces* perithecia with ascospores are rarely found on mulberry lesions under normal conditions, but are frequent if the lesions are kept wet. Perithecia are orange or red granular bodies, single or grouped.

The fact that a *Fusarium* causes root rot of ginseng has been known for 40 years (3), but a more precise identity of the fungus had not been established. We noted that our isolates bore cultural and morphological similarities to *F. solani* f. sp. *pisi*. The disease occurs mainly on fourth- or fifth-year roots, and is most conspicuous after heavy rains from July to September. Water-saturated soils seem to weaken ginseng roots and accelerate the disease. Round or irregular, light-brown lesions appear initially on lateral or main roots and may spread (Fig. 1-E), destroying all roots. Aboveground stems and leaves are then killed (Fig. 1-F). If diseased roots are dug and left aboveground, white mycelia of the *Fusarium* appear on lesion surfaces in a few days.

In a cooperative program between the USA and Japan from 1967 to 1970, we concentrated on (i) investigations of the host range of pea isolates from the collection at Berkeley and pea and mulberry isolates from the Japanese collection; (ii) mating studies of these isolates, in which the *Hypomyces* stage was formed; and (iii) the relationship of the ginseng root rot *Fusarium* to *F. solani* f. sp. *pisi*.

MATERIALS AND METHODS.–*Fusarium* (*Hypomyces*) *solani* App. & Wr. emend. Snyd. & Hans. f. sp. *pisi* (Jones) Snyd. & Hans. is composed of individuals that are self-sterile, interfertile hermaphrodites. In addition, unisexual males and females as well as neuters have been found; i.e., it is heterothallic in mating (compatibility) type, and hermaphroditic or unisexual (male or female) in sex. These mating types (+,–) and sex (M,F) were determined by crosses on potato-dextrose agar (PDA) with tester clones of known sex and mating types. All isolates were maintained by single-spore culture on PDA slants at room temperature (20 to 25 C) in diffuse daylight, or in daylight supplemented by 12 hr of fluorescent light. Crosses were made 20 to 30 days after single sporing by the pouring of a suspension of conidia in sterile water from one clone on a PDA slant containing another clone. The time required to obtain fertile perithecia varied from 1 to 3 months. Mating types and sex of isolates maintained in Berkeley and Japan were determined.

Pathogenicity to pea seedlings.–We made pathogenicity tests by inoculating emerging pea (*Pisum sativum* L.) seedlings of two American cultivars, Dwarf Telephone and Progress 9, and two Japanese cultivars, Sanjunichi-kinuzaya and Akabana-tsurunashi, growing in steam-treated soil, incorporated with spore suspensions from PDA slants. Four pots, each containing 8 to 10 plants, were used in testing each isolate, and the tests were repeated 2 to 3 times. Controls were noninoculated plants, all of which remained disease-free. Tests were terminated after 4 to 6 weeks. Plants were removed from the soil, washed, and graded as to degree of the fungal attack. This varied from severe (++++), with seedlings killed during the first 4 weeks after inoculation, to slight (+) or no (–) disease 6 weeks after inoculation.

Pathogenicity to mulberry branches.–The method of testing pathogenicity on mulberry (*Morus alba* L.), cultivar Kairyo-nezumigaeshi, was by means of a knife injury on the branch bark. In September or October, a peeling injury 2 × 2 mm was made on each branch in the field to which the test spores or hyphae were applied; the bark was restored to its original shape and painted with white petroleum jelly. The lesions were measured the following May. Six Berkeley pea isolates, three Japanese pea isolates, eight mulberry branch isolates of *F. solani* f. sp. *pisi*, and four ginseng root isolates were tested. Ten branches were used in testing each isolate. The test was repeated 3 times. Controls were injured, noninoculated branches. Virulence of the isolates was estimated by a ratio of length (mm) times width

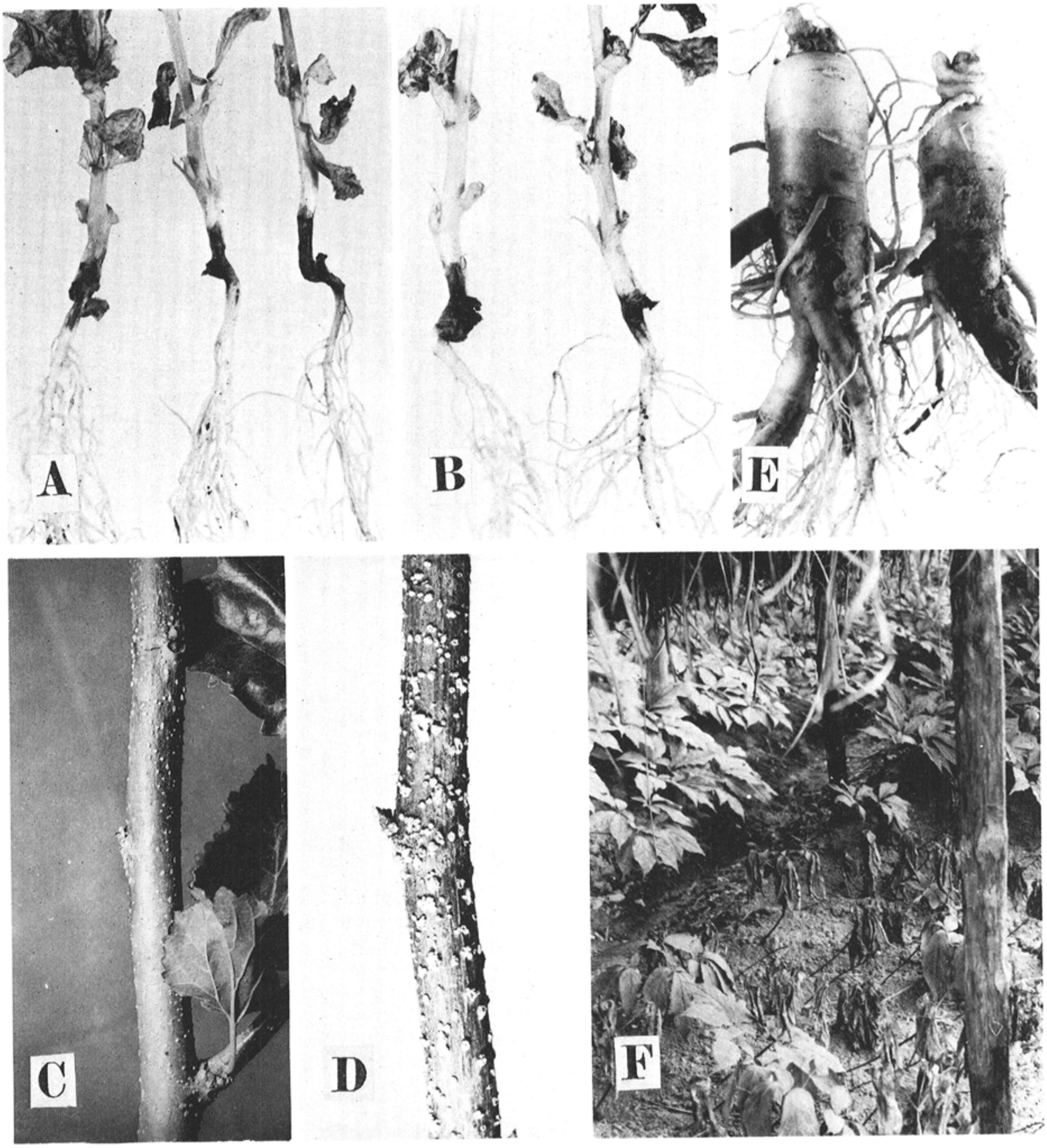

Fig. 1. A) Results of inoculation tests by a pea isolate of *Fusarium solani* f. sp. *pisi* to pea seedlings; and **B)** by a mulberry tree isolate of the same fungus. **C)** Field infection of a mulberry branch by *F. solani* f. sp. *pisi*; and **D)** sporodochia of the same fungus on an infected mulberry branch. **E)** Field infection of ginseng roots by f. sp. *pisi*; and **F)** aboveground symptoms in ginseng plants infected by the same fungus.

(mm) of lesions to the length times width of control injuries.

Pathogenicity to ginseng root.—Pathogenicity tests on ginseng (*Panax Ginseng* C. A. Mey) root consisted of planting healthy seeds or 2nd-year roots in pots of steam-treated soil infested with each isolate. The inoculum had been cultured on steam-sterilized bran, and 20 g of the bran was mixed into each 30 cm pot of soil. The pots were kept under shelter from sunlight, and the lower part of the pot was buried in soil. Results were measured the following year when the plants were dug. Three pots, each containing 20 seeds or six roots, were used in testing each isolate. Four pea isolates from the Berkeley collection and

TABLE 1. Mating types and sex of isolates of *Fusarium solani* f. sp. *pisi* in Berkeley and Japan laboratories

Host (disease)	Sex and[b] mating type	No. isolates
Pea (foot and root rot)		
Berkeley laboratory[a]	MF+	5
	MF–	1
	M+	5
	M–	3
	Unknown	9
Japan laboratory	MF+	1
	MF–	1
	M+	2
	Unknown	2
Mulberry tree (branch blight)	MF+	11
	MF–	13
	M+	4
	F+	5
	M–	4
	Unknown	3
Ginseng (root rot)	MF+	2
	MF–	2
	M+	2
	M–	1
	Unknown	11
Other hosts (saprophytic)	MF+	4
	MF–	1
	M+	2

[a] Including one English isolate and six South Australian isolates.
[b] M = male; F = female; + and – = mating types.

TABLE 2. Virulence of various isolates of *Fusarium solani* f. sp. *pisi* to pea seedlings

Pea cultivar	No. of isolates showing the virulence					
	++++[a]	+++	++	+	–	Total
Berkeley pea isolates						
Dwarf Telephone	6	8	4	2	2	22
Progress 9	10	6	2	1	2	21
Sanjunichi-kinuzaya	7	9	3	3	0	22
Akabana-tsurunashi	6	9	3	3	1	22
Japan pea isolates						
Dwarf Telephone	1	2	1	0	0	4
Sanjunichi-kinuzaya	2	1	1	0	0	4
Akabana-tsurunashi	1	2	1	0	0	4
Mulberry tree isolates						
Dwarf Telephone	2	3	4	2	0	11
Sanjunichi-kinuzaya	3	2	5	1	0	11
Akabana-tsurunashi	2	3	4	1	1	11
Ginseng isolates						
Dwarf Telephone	0	0	0	4	3	7
Sanjunichi-kinuzaya	0	0	0	4	3	7
Akabana-tsurunashi	0	0	0	3	4	7

[a] ++++, +++, ++, + indicate the degrees of virulence, and ++++ includes the killing of seedlings during the first 4 weeks after inoculation.

two from the Japanese collection, five mulberry isolates of *F. solani* f. sp. *pisi*, and four ginseng isolates were tested. Tests were repeated 2 to 3 times. Noninoculated controls remained disease-free. All isolates had predominantly 3-septate conidia, and were typically the f. sp. *pisi*.

RESULTS.–Some of the ginseng isolates produced *Hypomyces* perithecia and ascospores, not only by crossing with other ginseng isolates, but also with some *F. solani* f. sp. *pisi* clones. Thus, the causal *Fusarium* of ginseng root rot proved to be *F. solani* f. sp. *pisi*.

Table 1 shows the results obtained by all of the laboratory crosses made. In nature, the *Hypomyces* stage has never been found on diseased pea or ginseng, but it has been observed on diseased mulberry tree branches (cultivars Kairyo-nezumigaeshi and Ichinose) in several instances during the last decade in the Nagano Prefecture in June. Cultures derived from field-collected perithecia and single ascospores showed that various mating and sexual types, MF+, MF–, M+, M–, F+, F–, were the result of natural crosses.

Isolates (labeled "saprophytes" in Table 1) from several plants, including sweet potato, yam, and larch seedling roots, and from zelkova tree branches, were identified as *F. solani* f. sp. *pisi* as the result of mating tests, but proved weakly or nonpathogenic to the various hosts in pathogenicity tests. Most pea and mulberry tree isolates showed high virulence to the pea cultivars tested (Fig. 1-A, B); however, the ginseng root isolates showed weak or no virulence (Table 2).

Virulence to mulberry branches, measured by the ratio of the area of the lesion to the area of control branch injuries, rated 90 to 160 for most of the isolates. No substantial differences between averages of Berkeley pea isolates, Japanese pea, mulberry, or ginseng isolates occurred. It is interesting that ginseng isolates, having weak or no virulence to pea seedlings, showed a virulence to mulberry branches similar to the other isolates.

Results of the ginseng root pathogenicity test were not conclusive, but most of the isolates tested showed mild pathogenicity, in that some plants of each pot had main or lateral root lesions, and there was no appreciable difference resulting from the source of the isolates.

DISCUSSION.–Most of the described formae speciales of *Fusarium solani* are highly specialized as to host. This group includes *F. solani* f. sp. *cucurbitae* races 1 and 2 (14, 15); *F. solani* f. sp. *batatas* (14); *F. solani* f. sp. *phaseoli* (14); *F. solani* f. sp. *eumartii* (14); *F. solani* f. sp. *mori* (12); *F. solani* f. sp. *xanthoxyli* (13); and *F. solani* f. sp. *robiniae* (8). Only *F. solani* f. sp. *radicicola* (14) and *F. solani* f. sp. *pisi* are known to have wider host ranges. Although *F. solani* f. sp. *radicicola* can be isolated from lesions of many plants (potato tuber, tulip bulb, devil's tongue corm, etc.), it invades only through wounds and its pathogenicity is rather indefinite. Thus, *F. solani* f. sp. *pisi* can be regarded as a unique contrast to other formae speciales of *F. solani* in host range, because it invades pea, mulberry wood, ginseng, chickpea (5), and possibly other host plants. When properly mated, this fungus produced red perithecia in culture (7, 10) and was very similar to *Hypomyces (Fusarium) solani* f. sp. *cucurbitae* in appearance and sexual behavior (2), since it was heterothallic, had mating types (+,−), and through mutation developed unisexualism in respect to sex. This fungus did not mate with any clones of other formae speciales of *Fusarium solani*. We could identify various isolates of the present fungus by crossing them with one another, regardless of their host.

The results of crosses with tester clones of known mating type and sex showed that these isolates contain MF+, MF−, and some unisexual + and − types. However, in nature the *Hypomyces* stage of *F. solani* f. sp. *pisi* has been discovered only on the diseased branches of mulberry in the Nagano Prefecture of Japan and never on diseased pea, ginseng, or other plants. This fact may suggest that the origins of this fungus and the possible sources of new races in nature are in mulberry-growing regions, including Japan.

Most of the pea and mulberry isolates of f. sp. *pisi* showed high virulence to the three or four cultivars of pea with the exception of a few isolates, but ginseng root isolates showed weak or no virulence to the same pea cultivars. On the other hand, there were no remarkable differences concerning virulence to mulberry branches and ginseng root regardless of which hosts were the source of the isolate. In general, however, the results of inoculation tests on the branches of mulberry trees and the roots of ginseng were not as clear-cut as the seedling pea tests.

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