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Crown and Root Rot of Greenhouse Tomato Caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* in Korea

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Forty (40) isolates of *Fusarium oxysporum* isolated from wilting tomato plants at Buyeo of Korea in 1997 were inoculated to four tomato cultivars (Ponderosa, Okitsu 3, Walter, and Zuiken) to examine pathogenic reactions. Isolation rates of *F. oxysporum* f. sp. *lycopersici* (FOL) races 1 and 2, and *F. oxysporum* f. sp. *radicis-lycopersici* (FORL) were 3.5%, 24.5%, and 57.5%, respectively. Mycelial growth on potato-dextrose agar at different temperatures showed similar patterns, while optimum temperature for the three pathogens was 26°C. In the pathogenicity tests, however, the range of optimum temperature for disease development for FORL was between 15 and 20°C, while that for races 1 and 2 of FOL was between 25 and 30°C. In the host range studies, soybean, string bean and/or cucumber as well as tomato were susceptible to FORL, while races 1 and 2 of FOL were specifically pathogenic to tomato only. This suggests that host ranges of FORL and FOL differ significantly.

Keywords **:** forma specialis, *Fusarium oxysporum*, race, tomato.

Fusarium oxysporum has received considerable attention from plant pathologists over the past 80 years because of its ability to cause vascular wilt or root rot diseases on a wide range of plants. Despite the broad host range of the species, host specialization of individual isolates is more circumscribed. Isolates with the same or similar host ranges are assigned to a forma specialis, and more than 70 formae speciales have been described (Armstrong et al., 1981; Booth, 1971). More often than not, host range is restricted to a few plant species. For example, *F. oxysporum* f. sp. *lycopersici* (FOL) causes disease only in plants of the genus *Lycopersicon* (Rowe, 1980). However, some formae speciales have broader host ranges, such as *F. oxysporum* f. sp. *radicis-lycopersici* (FORL), which cause disease on hosts from several plant families, including tomato (*Lycopersicon esculentum* Mill.) in the greenhouse (Menzies et al., 1990; Rowe, 1980).

Crown and root rot of tomato caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* was first observed in greenhouses in the southern part of Japan in 1965 (Sato and Araki, 1974). Since then, it has become a major limiting factor in the production of greenhouse tomato in many countries (Jarvis et al., 1977; Nuter et al., 1978; Rowe et al., 1977). A similar disease caused by *F. oxysporum.* f. sp. *radicis-lycopersici* (FORL) in the rockwool culture system was reported in Korea (Lee et al., 1994).

At first, the causal fungus was identified as a new race (J3) of *F. oxysporum* Schlecht. f. sp. *lycopersici* Snyd. & Hans. which causes Fusarium wilt of tomato (Sato and Araki, 1974; Yamamoto et al., 1974). However, Jarvis and Shoemaker (1978) pointed out that the causal agent was not a new race of *F. oxysporum* f. sp. *lycopersici* but a new forma specialis of *F. oxysporum,* and have designated the fungus as *F. oxysporum* Schlecht. f. sp. *radicis-lycopersici* Jarvis Shoemaker. Their conclusions have been based on the following characteristics:

1) The FORL pathogen has distinctly different symptoms from those caused by FOL. Disease symptoms in mature crops caused by FORL are those of root and basal stalk rot rather than vascular wilt.

2) Crown and root rot disease occurs at cool (18°C) soil temperatures (Jarvis and Thorpe, 1976; Sato and Araki, 1974; Sonoda, 1976; Yamamoto et al., 1974), while that caused by FOL is most severe at soil temperatures of about 27°C.

3) The host range of FORL is larger than FOL (Rowe, 1980; Menzies et al., 1990). When Rowe (1980) tested pathogenicity to 17 plant species by inoculating different isolates of the crown rot organism, various species of the family Leguminosae, as well as *L. esculentum*, were infected. However, *F. oxysporum* f. sp. *lycopersici* is specific only to *Lycopersicon* spp.

The objectives of this study were to: 1) confirm whether the collected isolates were races 1 and 2 of *F. oxysporum* f.

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sp. *lycopersici* or a new forma specialis of *F. oxysporum* as *F. oxysporum* f. sp. *radicis-lycopersici*; 2) characterize the optimum temperature of these pathogens for mycelium growth and disease development; and 3) determine the host range of these pathogens to six different plant species.

Materials and Methods

Collection and storage of isolates. A total of 47 isolates of *F. oxysporum* were used in this pathogenicity test. Seven isolates were supplied by W. R. Jarvis and T. Katan as comparison isolates, while 40 isolates were collected from wilting tomato plants in greenhouses in Buyeo, Korea. Stems and roots from symptomatic plants were washed with tap water. After removal of the stem cortex, small pieces of chocolate brown vascular tissue were surface-sterilized in 0.5% NaOCl for 30-60 sec, then placed in petri plates containing 2% water agar or acidified potato-dextrose agar (APDA) containing 2 ml of 25% lactic acid per liter or Komada's agar (KA), as a selective medium for *F. oxysporum*. Plates were incubated in a laboratory incubator at 26±1°C. After 2-3 days, colonies of *F. oxysporum* were sub-cultured onto non-acidified PDA. Monoconidial isolates were prepared, then microspore suspensions of these were added to sterile soil in small vials and incubated at 26°C for 10-14 days. For long-term storage, soil cultures were kept at 5°C.

Inoculation of tomato cultivars. To compare the pathogenic reactions of *F. oxysporum* isolates, four tomato cultivars were selected. Cultivar Ponderosa was the susceptible control, while cv. Okitsu 3 (susceptible to FORL and FOL race 2, but resistant to FOL race 1) and cv. Walter (resistant to both races of FOL, but susceptible to FORL) were used to identify isolates of FOL (Yamamoto et al., 1974; Kuniyasu, 1990). The Japanese breeding cultivar Zuiken F1 (Sakada Seeds), which is resistant to FORL, was also used to distinguish from FOL isolates (Table 1).

Inoculum of isolates consisted of microspores produced in potato-dextrose both by shake culture at 25°C for 3-4 days. Microspore suspensions were filtered through cheesecloth and final concentrations were adjusted to 10^7 spores/ml. The roots of 20- to 30-day-old tomato seedlings, which were steam-sterilized in greenhouse soil, were carefully uprooted and freed from soil by immersion in water and gentle shaking. Then they were dipped into a microspore suspension for 10-30 min and replanted to pots (9 cm in diameter). Inoculated plants were grown in a room bench at 22-25°C. After 3 weeks, they were uprooted and the lower stem and tap root were longitudinally sectioned for examination of internal tissues. Each plant was rated on a scale of 0-4 as follows: 0 = healthy plants; 1 = < 25% vascular discoloration; 2 = 26-50% vascular discoloration; 3 = wilting with 51-75% vascular discoloration; and 4 = 76-100% vascular discoloration or death.

Effect of temperature on mycelial growth of the pathogen. Petri dish media (8.7 cm in diameter) containing 20 ml of PDA were inoculated centrally with 5-mm plugs taken from the periphery of young cultures of FOL races 1 and 2 and FORL isolates with three replicates. The plates were incubated at 18, 22, 24, 26, 30, and 32°C for 6 days in the dark, and colony diameter was measured in two orthogonal directions.

Effect of temperature on disease development. To test the effect of temperature on disease development, tomato plants of cv. Ponderosa (susceptible to FORL and the two races of FOL) were grown. Ten seedlings at the four-true-leaf stage were each inoculated with isolates of FORL and races 1 and 2 of FOL using the root dip method, and then the inoculated plants were grown in a room bench at 15, 20, 25, and 30°C under fluorescent light for 14 h. After 2 weeks, the plants were uprooted and the disease severity was determined according to disease index with a scale of 0-4.

Host range tests. Host range studies were made to differentiate FORL isolates from FOL races 1 and 2, which are specific to tomato. Six crop plants from four botanical families were selected for testing. Each ten seedlings at the four-true-leaf stage of tomato (cvs. Ponderosa, Okitsu 3, and Walter), pepper (cv. Luck red), soybean, string bean, cucumber (cv. Tenma), and chard were inoculated by using the root dip method, as described above. After inoculation, seedlings were incubated at 22-25°C under supplemental fluorescent light for 14 h. Final observations of disease development on seedlings were made 30 days after inoculation.

Table 1. Susceptibility of four tomato cultivars against different races of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and *F. oxysporum* f. sp. *radicis-lycopersici* (FORL)

Tomato cultivars	Isolates		
	FOL		FORL
	Race 1	Race 2	
Ponderosa	S[a]	S	S
Okitsu 3	R	S	S
Walter	R	R	S
Zuiken	R	S	R

[a] Plant reaction: S = susceptible, R = resistant.

Results

Inoculation of tomato cultivars. For the pathogenicity test, four tomato cultivars were inoculated with spore suspension of 47 isolates. Seven of the 47 isolates were supplied by W. R. Jarvis of Canada and T. Katan of Israel as comparison pathogen. The total collected isolates were clearly distinguished as races 1 and 2 of FOL, or FORL based on the reaction of the four tomato cultivars. In particular, FORL isolates were distinguished from those of FOL when the isolates were compared with cv. Walter which was susceptible to only FORL, and cv. Zuiken which was resistant to FORL but susceptible to race 2 of FOL (Table 1).

The pathogenicity of only 17 of the 47 isolates were presented in Table 2. The isolate of TF-428 showed pathogenicity to cv. Ponderosa, while TF-343, TF-367, and TF-377 showed pathogenicity against cvs. Ponderosa, Okitsu 3, and Zuiken. On the other hand, TF-346, TF-348, TF-358, TF-

Table 2. Pathogenicity of *Fusarium oxysporum* isolates from wilted tomato plants to four tomato cultivars

Isolate	Origin		Disease index [a]				f. sp. and race [b]
	Tomato	Location	Ponderosa	Okisu 3	Walter	Zuiken	
TF-428	Minicarol	Buyeo, Korea	2.3	0.7	0.5	0.6	FOL 1
TF-343	Minicarol	Buyeo, Korea	3.8	4.0	0.6	3.5	FOL 2
TF-367	Momotaro	Buyeo, Korea	2.7	4.0	0.7	2.7	FOL 2
TF-377	Sanchery	Buyeo, Korea	4.0	4.0	0.1	2.9	FOL 2
TF-346	Minicarol	Buyeo, Korea	3.1	4.0	3.7	0.0	FORL
TF-348	Momotaro	Buyeo, Korea	3.9	4.0	3.1	0.2	FORL
TF-358	Momotaro	Buyeo, Korea	4.0	4.0	3.8	0.3	FORL
TF-381	Sanchery	Buyeo, Korea	2.5	2.8	2.4	0.0	FORL
TF-403	Momotaro	Buyeo, Korea	3.0	3.5	2.5	0.1	FORL
TF-340	Minicarol	Buyeo, Korea	0.5	0.3	0.3	0.4	NON
# 38		Canada	2.7	0.2	0.5	0.5	FOL 1
FOL-1		Israel	3.7	0.0	0.2	0.2	FOL 1
FOL-481D		Israel	4.0	3.0	0.0	3.7	FOL 2
FORL-C85		Israel	3.3	4.0	3.3	0.7	FORL
FORL-809		Israel	3.3	2.8	3.5	0.4	FORL
# 82		Canada	1.4	2.0	1.2	0.3	FORL
# 153		Canada	2.2	1.8	2.3	0.0	FORL

[a] Disease index: 0 = no symptom, 1 = <25% vascular discoloration, 2 = 26-50% vascular discoloration, 3 = wilting with 51-75% vascular discoloration, 4 = 76-100% vascular discoloration or death.
[b] FOL 1 = *Fusarium oxysporum* f. sp. *lycopersici* race 1, FOL 2 = *Fusarium oxysporum* f. sp. *lycopersici* race 2, FORL = *Fusarium oxysporum* f. sp. *radicis- lycopersic*, NON = non-pathogenicity.

381, and TF-403 showed pathogenicity against cvs. Ponderosa, Okitsu 3, and Walter. Accordingly, based on the pathogenicity reactions, these isolates were identified as FOL races 1 and 2, and FORL. Meanwhile, their isolation rates were 3.5, 24.5, and 57.5%, respectively.

Effect of temperature on mycelial growth of the pathogen. Colonies grew well on PDA media between 18 and 32°C regime, with the optimum temperature at 26°C for all tested isolates (Fig. 1).

Effect of temperature on disease development. The range of optimum temperature for disease development by FORL pathogen was between 15 and 20°C, as against 20-30°C for races 1 and 2 of FOL pathogens (Fig. 2).

Host range tests. According to inoculation studies, tomato, soybean, string bean, and/or cucumber were susceptible to TF-346 and TF-381 isolates of FORL pathogens, while the FOL races 1 and 2 were host specific to susceptible culti-

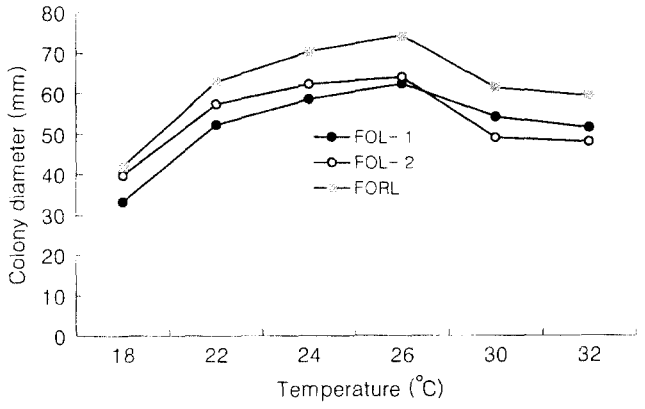

Fig. 1. Mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) races 1 and 2, and *F. oxysporum* f. sp. *radicis-lycopersici* (FORL) on PDA at different temperatures. Colony diameter was measured 6 days after culture.

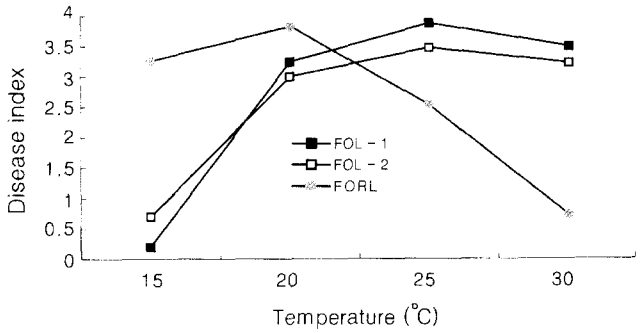

Fig. 2. Pathogenicity of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and *F. oxysporum* f. sp. *radicis-lycopersici* (FORL) to cv. Ponderosa at four different temperatures. Disease index is 0-4 scale: 0 = no symptoms, 1 = <25% vascular discoloration, 2 = 26-50% vascular discoloration, 3 = wilting with 51-75% vascular discoloration, 4 = 76-100% vascular discoloration or death.

Table 3. Host range of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and *F. oxysporum* f. sp. *radicis-lycopersici* (FORL)

Plant	Disease severity[a]			
	FOL		FORL	
	Race 1 (TF-428)	Race 2 (TF-377)	(TF-346)	(TF-381)
Tomato cv. Ponderosa	+	+	+	+
Tomato cv. Okitsu 3	−	+	+	+
Tomato cv. Walter	−	−	+	+
Pepper cv. Luck red	−	−	−	−
Soybean	−	−	+	±
String bean	−	−	±	±
Cucumber cv. Tengma	−	−	+	+
Chard	−	−	±	−

[a] Disease severity, + = wilting with vascular discoloration, ± = vascular discoloration, − = no discoloration.

vars of tomato (Table 3). Earlier host range studies by Rowe (1980) and Menzies et al. (1990) showed that some isolates of FORL were highly virulent to soybean, string bean, cucumber, and/or chard.

Discussion

Weimer (1944) described a root rot of lupine and named the pathogen *F. oxysporum* f. sp. *radicis-lupini* to differentiate it from *F. oxysporum* f. sp. *lupini* which caused vascular wilt of lupine. Armstrong et al. (1968), in fact, excluded f. sp. *radicis-lupini* from their list of forma speciales of *F. oxysporum* because it did not cause vascular wilt. Gordon (1965), however, broadened the concept to include pathogenic forms not causing vascular wilts and again included f. sp. *radicis-lupini* in the list. Although most pathogenic forms of *F. oxysporum* cause vascular wilts, this species has also caused root, crown, and bulb rots (Abawi and Lorbeer, 1972; Bloomberg, 1973; Joffe, 1974; Warren et al., 1973).

Inoculation with 47 isolates of *F. oxysporum* isolated from wilting tomato plants in greenhouses was carried out to differentiate races and forma specialis of *F. oxysporum* by using four tomato cultivars according to the method of Yamamoto et al. (1974). However, while Yamamoto et al. used only three cultivars (cvs. Ponderosa, Okitsu 3, and Walter), this study used one more (cv. Zuiken) in addition to the three (Table 1). Using these tomato cultivars, the differences of pathogenicity among FORL and FOL races 1 and 2 were confirmed. The isolation rate of FORL, of which cvs. Ponderosa, Okitsu 3, and Walter were susceptible while cv. Zuiken was resistant to, was the highest at 57.5%. This result is very interesting because the FORL pathogen has not been reported yet in tomato growing areas in Korea, except for the report of tomato stem girdling and necrosis at the rockwool line by Lee et al. (1994). In Japan, Yamamoto et al. (1974) described the crown rot of greenhouse tomato in southern Japan as caused by a new race (J3) of *F. oxysporum* f. sp. *lycopersici*. This was based on the pathogenicity of the new race to cv. Walter which is resistant to both known races of FOL. Since then, there has been some disagreement with the designation of this pathogen as a new race of FOL because the symptoms are not those typical of vascular wilt but those of crown rot disease (Jarvis et al., 1977; Nutter et al., 1978; Rowe et al., 1977).

In this study, the typical characteristics of a new forma specialis (FORL) were confirmed. Symptoms of mature crops in the greenhouse include chocolate-brown cortical rot and girdling at soil level. The fungus can be isolated only from within a few millimeters of the edge of the discoloration area, whereas, FOL can be isolated from higher part of the wilted plants. This result is consistent with Jarvis previous report (1988). The reaction of four tomato cultivars to the pathogen in the pathogenicity test was quite distinct, as presented in Table 2. Although the mycelial growth was highest at 26°C for all isolates, optimum temperature for disease development was about 15-20°C, in contrast with the 25-30°C optimum for FOL. This result concurs with that of other researchers (Jarvis and Thorpe, 1976; Sato and Araki, 1974; Yamamoto et al., 1974). It was also confirmed that FORL has a broad host range including string bean, soybean, cucumber, and chard, as well as tomato. The crop damage caused by FORL in Korea is expected to increase with increasing acreage of greenhouse year by year.

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