

Root and Stem Rot of Cucumber Caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* f. sp. nov.

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ABSTRACT

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Root and stem rot of cucumber (*Cucumis sativus*) caused by a new *Fusarium oxysporum* forma specialis is reported for the first time. Of 18 cultivated species from six different botanical families that were artificially inoculated, two species of Cucurbitaceae—melon (*C. melo*) and sponge gourd (*Luffa aegyptiaca*)—showed root and stem rot similar to that caused by isolates of *F. oxysporum* that attack cucumber. Optimum temperature for disease development was close to 17°C. Disease symptoms and pathogenicity tests confirm that a new forma specialis of *F. oxysporum* is the incitant of the disease. The author proposes to name this forma specialis as *F. oxysporum* f. sp. *radicis-cucumerinum* f. sp. nov.

During the 1989–90 crop season a severe root and stem rot of cucumber (*Cucumis sativus* L.) occurred in a few plastic houses in the Ierapetra area of Lasithi, Crete, Greece. Since then, the disease has spread to most of the other cucumber-growing areas of Crete, and during the crop seasons of 1992–93 and 1993–94 it caused severe losses in most of the south-eastern cucumber-growing areas on the island.

Symptomatology. Symptoms first appear in late autumn on plants about 1 month old. Collar rot and later hypocotyl rot develop, usually on one side of the stem, which ranges in color from very light green to amber and brown. Progressively, the hypocotyl rot becomes more severe and a white fungal growth may appear on the affected tissue (Fig. 1A,B). The primary root and several secondary roots rot and the basal portion of the stem shows a vascular brown discoloration. Plants with these symptoms have a stunted growth, and wilt and die within a few weeks. Plants can also wilt suddenly and die without prior hypocotyl rotting.

Although young plants can be killed, usually plants reach cropping size and symptoms do not appear until first-fruiting stage. Adult plants then undergo slow wilting with a progressive yellowing (Fig. 1C). Infected plants with heavy fruit loads wilt on sunny days but may recover at night. However, they die after repeated wilting. These symptoms usually follow a unilateral cortical rot with a longitudinal

canker at the hypocotyl that may extend upward for 20 to 40 cm, and downward to the root system (Fig. 1D). On the stem a vascular brown discoloration may appear, extending for 40 to 200 cm above the soil line. Primary, secondary, and tertiary roots have brown lesions, many confluent with hypocotyl lesions. Isolated unilateral cracks with rots varying in length from 5 to 15 cm or more, usually with white growth of the pathogen, might also appear on the upper portion of the stem. Under humid weather an orange mass of *Fusarium*-like conidia appear on the necrotic zone of the stem. During the winter, when plant vigor within greenhouses is reduced due to unfavorable microclimatic conditions (reduced illumination and average air temperature lower than 15°C), the disease progresses fast, causing severe damage.

Initial observations placed the fungus in *Fusarium oxysporum* Schlechtend.:Fr. *Fusarium* wilt, caused by *F. oxysporum* f. sp. *cucumerinum* J. H. Owen races 1, 2, and 3 (2,6,7) is a common disease of cucumber in many parts of the world (8). However, the symptoms of the present disease suggested some anomaly warranting additional investigations. The purpose of the present study was to determine the host range of the pathogen and to characterize the strains causing root and stem rot of cucumber.

MATERIALS AND METHODS

Causal agent. Small pieces of affected vascular tissue taken from the hypocotyl area were plated on acidified potato-dextrose agar (APDA) containing 4.5 ml of 25% lactic acid per liter (final pH 4.0). The plates were incubated at 24°C in the dark for 4 days.

Isolates. Three single-spore isolates designated as AFu-4(C), AFu-7(A), and AF-1(a) of the root and stem rot fungus,

obtained from diseased cucumber plants from three commercial plastic houses in the Ierapetra area, Lasithi, Crete, were used for artificial inoculations and for studies on morphological and cultural characteristics of the pathogen.

Effect of temperature on mycelial growth of the pathogen. Petri dishes (9 cm diameter) containing 20 ml of APDA were inoculated centrally with 5-mm plugs taken from the periphery of young cultures of isolate AF-1(a) of the pathogen in four replicates. The plates were incubated at 8, 11, 14, 17, 20, 23, 25, 27, 29, and 33°C for 6 days in the dark and colony diameter was measured every 2 days in two orthogonal directions. Petri dishes of APDA inoculated similarly with PHW 231 of *F. oxysporum* f. sp. *cucumerinum* (race 1) (P. H. Williams, Department of Plant Pathology, University of Wisconsin-Madison) were also included in the test for comparison. The test was done twice.

Inoculum preparation. Inoculum was prepared by growing the fungus in potato-dextrose broth (PDB) in 200-ml Erlenmeyer flasks in a rotary shaker for 7 days, at 20°C in the dark. The suspension was then filtered through a double layer of nonsterile cheesecloth to remove mycelial fragments, centrifuged at 3,000 × g for 10 min and then resuspended in distilled water.

Plant inoculation. Two inoculation procedures were employed. In the first procedure, seedlings at the one-true-leaf stage and plants at the 10-true-leaf stage were removed from the organic substrate Belplanto (Klasmann-Deilmann GmbH, Geeste, Germany) and the roots washed with tap water. Root tips were trimmed from the root ball with a scissors and the roots were dipped in a spore suspension (10⁶ spores/ml) of the test fungus for 30 min. Seedlings were then transplanted in sterile organic substrate Belplanto and kept in growth chambers at 24°C with a 12-h photoperiod. Older plants inoculated similarly were grown in a glasshouse with temperatures fluctuating from 10 to 25°C. Plants treated similarly and dipped in tap water served as control.

In the second inoculation procedure, plants at the three- to four-true-leaf stage were removed from the organic substrate Belplanto, roots were washed with tap water, and plants were transplanted in sterile substrate Belplanto, previously infested with a spore suspension adjusted to 10⁶ spores/ml (1 part inoculum/10 parts

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substrate, vol/vol). Plants were kept in growth chambers at 24°C with a 12-h photoperiod.

Host range of the pathogen. To determine the host range of the pathogen, three different experiments were conducted.

In the first experiment, 15 seedlings of each potential host, at the one-true-leaf stage, were inoculated with the new pathogen using the root dip method and then incubated in a growth chamber for disease development, as described earlier. Final observation of disease development was made 30 days after inoculation.

In the second experiment, 15 plants of each of all test hosts, at the three- to four-true-leaf stage, were planted in an organic substrate Belplanto infested with the new pathogen. Final observation of disease development was made 30 days after inoculation.

In the third experiment, 10 plants of each potential host, at the 10-true-leaf stage, were inoculated with the new pathogen using the root dip method. Then plants were transplanted in pots with 3 liters of infested organic substrate Belplanto and kept in a glasshouse. Final observation of disease development was made 60 days after inoculation. All three experiments were done twice.

Pathogenicity tests of various formae speciales of *Fusarium oxysporum* obtained from cucurbit species. In pathogenicity tests using the root dip method, the new pathogen was compared with five formae speciales of *F. oxysporum* attacking various cucurbits (Table 1). Although the new pathogen causes symptoms similar to those described for *F. solani* (Mart.) Sacc. f. sp. *cucurbitae* W. C. Snyder & H. N. Hans., the latter was not included in the tests since quarantine considerations pre-

vented us from importing it into Greece.

Seedlings at the one-true-leaf stage and at the three- to four-true-leaf stage and plants at the 10-true-leaf stage of *Cucumis sativus* L., *C. melo* L., *Citrullus lanatus* (Thunb.) Matsum. & Nakai, *Lagenaria vulgaris* Ser., *Luffa aegyptiaca* Mill., *Momordica charantia* L., and *Benincasa hispida* (Thunb.) Cogn. were inoculated with the new pathogen or with one of the formae speciales of *F. oxysporum* attacking various cucurbits (Table 1), as described earlier. After inoculation, seedlings were incubated in a growth chamber and older plants in a glasshouse for disease development. Final observations of disease development on seedling and older plants were made 30 and 60 days after inoculation, respectively.

Tests for comparison of the new pathogen with races 1, 2, and 3 of *F. oxysporum* f. sp. *cucumerinum*. In pathogenicity tests using the root dip method, the new pathogen was compared with the isolates #16416 (race 1), #36330 (race 2) and #36332 (race 3) of *F. oxysporum* f. sp. *cucumerinum* (Table 1) obtained from the American Type Culture Collection (ATCC), by using plants, at the three-true-leaf stage, of the race-differential cvs. Ashley, Chipper, MSU-8519, and PI-390265 (2), as well as of the cvs. Straight-8, which is susceptible, and SMR-18 and Santo F., which are resistant, to races 1, 2, and 3 of *F. oxysporum* f. sp. *cucumerinum*, respectively (9,10). Final observation of disease development was made 50 days after inoculation.

Effect of temperature on disease development. To test the effect of temperature on disease development, cucumber plants of the local cv. Knossos (susceptible to the new pathogen and all three races of *F. oxysporum* f. sp. *cucumerinum*) were planted in an organic substrate Belplanto infested with the new pathogen, as described earlier. Plants were incubated in growth chambers at 17, 23, 29, and 35°C for disease development, in 15 replicates per temperature. Final observation of disease development was made 40 days after inoculation. Plants planted in organic substrate Belplanto that had been infested with the isolate PHW 231 of *F. oxysporum* f. sp. *cucumerinum* (race 1) and then treated similarly were also included in the test for comparison. The test was done three times.

RESULTS

Causal agent. Microconidia and macroconidia on sporodochia of *F. oxysporum* were always observed on the hypocotyl lesions. The same fungus was consistently isolated from numerous samples taken from different plastic houses.

Morphological and cultural characteristics of the pathogen. The colonies of the fungus on APDA were white with prolific aerial mycelium. At 23°C, the colo-

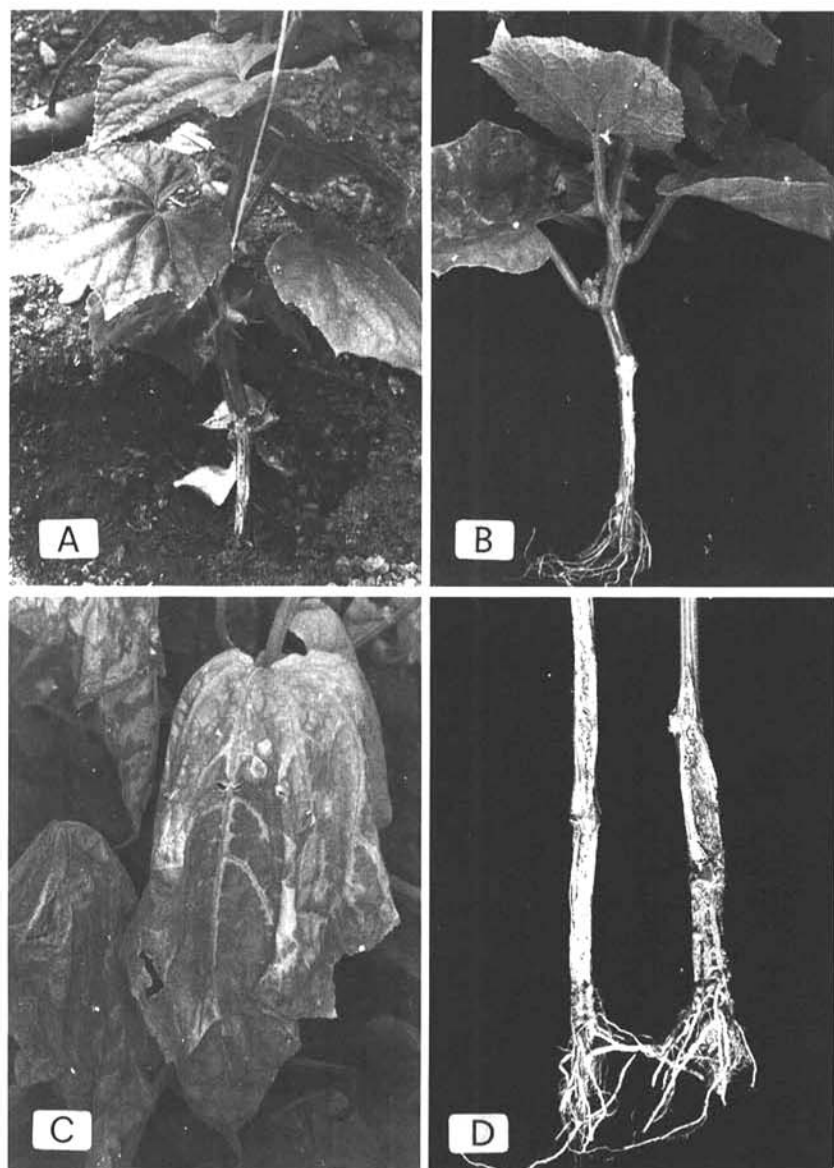


Fig. 1. Symptoms on cucumber plants caused by *Fusarium oxysporum* f. sp. *radialis-cucumerinum*. (A, B) Young plants with stem rot; white fungal growth of the pathogen on the hypocotyl. (C) Yellowing and wilting of leaves of older plants. (D) Cracks and rot on root and stem of older plants.

nies were white with prolific aerial mycelium. Numerous green-blue sclerotia and large numbers of macroconidia on sporodochia in a small area around the plug were present. At 27 to 33°C, the colonies were white with no sclerotia, but with progressively increasing areas of slimy sporodochia with numerous macroconidia around the plug as the temperature increased. At 35°C, slimy sporodochia occupied the whole dish. At 20°C, the lower surface of the colony was orange-yellow, with a small violet area around the plug. The growth of the colonies on APDA was rapid at 20°C (0.8 cm/day).

Microconidia and macroconidia were formed abundantly on lesions of the hypocotyl. Microconidia were hyaline with the following shapes: oval (7%) 6.3 ± 1.45 ($4.8-9.7$) \times 3.0 ± 0.48 ($2.4-3.6$) μm ; slightly curved (28%) 9.4 ± 2.18 ($6.1-15.7$) \times 2.9 ± 0.48 ($2.4-4.4$) μm ; cylindrical (65%) 8.2 ± 2.18 ($4.3-12.1$) \times 2.7 ± 0.48 ($1.9-3.6$) μm (average of 325 conidia). Macroconidia were hyaline, fusiform, slightly to strongly curved, foot cells generally inconspicuous, and they ranged from 1 to 4 septate in the following frequency: 1 septate (12%) $24.4-31.5$ ($24.4-31.5$) \times 3.4 ± 0.73 ($2.4-4.8$) μm ; 2 septate (3%) 28.6 ± 6.53 ($16.9-36.3$) \times 4.4 ± 1.21 ($2.4-5.3$) μm ; 3 septate (84%) 33.2 ± 3.15 ($21.8-41.1$) \times 4.6 ± 0.48 ($3.6-6.1$) μm ; 4 septate (1%) 38.2 ± 2.66 ($36.3-39.9$) \times 5.4 ± 0.97 ($4.8-6.1$) μm (average of 200 conidia).

On APDA at 23°C, microconidia were abundant on short conidiophores, one-celled, oval, slightly curved, cylindrical, spherical, 8.3 ± 3.59 ($2.4-21.8$) \times 2.8 ± 0.52 ($2.4-4.4$) μm (average of 300 conidia). Macroconidia were hyaline, fusiform, slightly to strongly curved, 1 to 4 septate, 30.1 ± 4.58 ($19.3-41.1$) \times 4.1 ± 0.69 ($2.4-4.8$) μm (average of 300 conidia). Chlamydo-spores were nonseptate, spherical or elliptic, hyaline to light brown, smooth, terminal or intercalary, 8.7 ± 1.91 ($4.8-14.5$) μm (average of 100 chlamydo-spores).

The morphological and cultural characteristics of the fungus in vivo and in vitro are in agreement with the published descriptions of *Fusarium oxysporum* Schlechtend.:Fr. (3). The identity of the pathogen was confirmed by Keith Seifert (National Identification Service, Economic Fungi, Centre for Land and Biological Resources Research, Ottawa, Ontario, Canada).

Effect of temperature on mycelial growth of the pathogen. Colonies developed on APDA between 8 and 33°C, with optimum development at 27°C.

Host range of the pathogen. In the first experiment, all plants of *C. sativus*, *C. melo*, *C. lanatus*, *L. vulgaris*, and *L. aegyptiaca* were dead 15 days after inoculation, while seedlings of *Cucurbita pepo* L., *C. ficifolia* Bouche, *C. moschata* (Duchesne) Duchesne ex Poir., *M. charantia*,

B. hispida, *Lycopersicon esculentum* Mill., *Solanum melongena* L., *Capsicum annuum* L., *Phaseolus vulgaris* L., *Brassica oleracea* L. var. *capitata*, *Daucus carota* L., *Petroselinum crispum* (Mill.) Nym. ex A. W. Hill, and *Beta vulgaris* L. showed no symptoms. They also remained symptomless 30 days after inoculation.

In the second experiment, results were similar with those of the first experiment with the exception that *C. lanatus* and *L. vulgaris* were symptomless.

In the third experiment, plants of *C. sativus*, *C. melo*, and *L. aegyptiaca* showed, 1 month after inoculation, cortical rot at the hypocotyl area covered with an orange mass of *Fusarium* conidia. One month later all plants were dead, while the remaining plants of *C. lanatus*, *C. ficifolia*, *C. moschata*, *L. vulgaris*, *L. esculentum*, *S. melongena*, *C. annuum*, and *P. vulgaris* were symptomless.

In all three experiments *F. oxysporum* was reisolated from inoculated plants.

Pathogenicity tests of various formae speciales of *F. oxysporum* obtained from cucurbit species. In both seedlings and older plants, plant species were attacked only by their respective forma specialis. *Cucumis sativus* was infected only by *F. oxysporum* f. sp. *cucumerinum* and the new pathogen, but the symptoms they caused were different. *Fusarium oxysporum* f. sp. *cucumerinum* caused yellowing, wilting, and vascular discoloration of the stem without any root or stem rot. In contrast, the new pathogen caused a severe root and hypocotyl rot of the plants accompanied by a vascular discoloration in the stem.

Tests for comparison of the pathogen with races 1, 2, and 3 of *F. oxysporum* f. sp. *cucumerinum*. All three races of *F. oxysporum* f. sp. *cucumerinum* caused

Table 1. Comparison of five formae speciales and three races of *Fusarium oxysporum* f. sp. *cucumerinum* with *F. oxysporum* f. sp. *radicis-cucumerinum*

Forma specialis and race	Plant source	Origin
<i>niveum</i> (E. F. Sm.) W. C. Snyder & H. N. Hans., race 2	<i>Citrullus lanatus</i> (Thunb.) Matsum. & Nakai.	D. Netzer, Volcani Center, Israel
<i>melonis</i> W. C. Snyder & H. N. Hans., race 1	<i>Cucumis melo</i> L.	P. Mas, INRA, France
<i>momordicae</i> Sun & Huang	<i>Momordica charantia</i> L.	S.K. Sun, National Chung Hsing University, Taiwan
<i>cucumerinum</i> J. H. Owen, race 1	<i>Cucumis sativus</i> L.	ATCC and P.H. Williams, University of Wisconsin-Madison
<i>cucumerinum</i> J. H. Owen race 2	<i>Cucumis sativus</i> L.	ATCC
<i>cucumerinum</i> J. H. Owen race 3	<i>Cucumis sativus</i> L.	ATCC

Table 2. Disease reaction of race differential and other cucumber cultivars to races 1, 2, and 3 of *Fusarium oxysporum* f. sp. *cucumerinum* and to isolates AFu-4(C) and AFu-7(A) of *F. oxysporum* f. sp. *radicis-cucumerinum* in a growth chamber experiment

Cultivar	<i>F. oxysporum</i> f. sp. <i>cucumerinum</i>			<i>F. oxysporum</i> f. sp. <i>radicis-cucumerinum</i>	
	#16416 (race 1)	#36330 (race 2)	#36332 (race 3)	AFu-4(C)	AFu-7(A)
Ashley	W ^a	W	W	R	R
Straight-8	W	W	W	R	R
Chipper	W	W	H	R	R
MSU-8519	±	H	H	±	R
SMR-18	H	H	H	R	±
PI-390265	H*	H*	H*	H*	H*
Santo F ₁	H	H	H	R	R

^a W = wilting with vascular discoloration on root and stem; R = cortical rot on root and stem; H = healthy plants, no symptoms; H* = no wilting or cortical rot, mostly with light vascular discoloration on root and lower part of the stem; ± = approximately 50% of the plants diseased and 50% healthy.

Table 3. Effect of temperature on root and stem rot (RSR) of cucumber cv. Knossos caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* vs. *Fusarium wilt* (FW) caused by *F. oxysporum* f. sp. *cucumerinum* race 1 in a growth chamber experiment

Observation date (days after inoculation)	Total number of dead plants ^a							
	17°C		23°C		29°C		35°C	
	RSR	FW	RSR	FW	RSR	FW	RSR	FW
20	3	0	6	0	0	15	0	4
30	15	0	10	7	0	15	0	7
40	15	0	10	15	0	15	0	11

^a Total number of dead plants from 15 artificially inoculated plants per pathogen and temperature.

yellowing, wilting, and vascular discoloration of the susceptible race differential plants and other cultivars (Table 2). They colonized the root cortex only sparsely and entered the xylem vessels. In contrast, the new pathogen caused cortical rot in the basal stem tissue and roots of cvs. Ashley, Straight-8, Chipper, MSU-8519, SMR-18, and Santo, but not in PI-390265, which showed only a light vascular discoloration extending up to two nodes above the hypocotyl.

Effect of temperature on disease development. Optimum temperature for disease development was close to 17°C, in contrast with *Fusarium* wilt caused by *F. oxysporum* f. sp. *cucumerinum* race 1, which was most severe at temperatures close to 29°C (Table 3).

DISCUSSION

Of the 18 plant species from the families Chenopodiaceae, Cruciferae, Cucurbitaceae, Leguminosae, Solanaceae, and Umbelliferae that were artificially inoculated with the new pathogen, at the three- and 10-leaf stages, only *C. sativus*, *C. melo*, and *L. aegyptiaca* of the Cucurbitaceae showed root and stem rot similar to that caused by isolates of *F. oxysporum* that attack cucumber. The fungus entered the cortical tissues of roots and caused a brown lesion that extended into the vascular system. Vascular discoloration extended upward to the stem.

The new disease is essentially one that occurs at cool soil temperatures (optimum temperature close to 17°C), thus differing from the wilt caused by *F. oxysporum* f. sp. *cucumerinum*, which is most severe at high soil temperatures (optimum temperature close to 29°C).

Infections of cucumber by *Fusarium* spp. are very common, but have always been caused by *F. oxysporum* f. sp. *cucumerinum* and *F. s. f. sp. cucurbitae* (8). The new pathogen clearly is not a typical vascular wilt pathogen as are races 1, 2, and 3 of *F. oxysporum* f. sp. *cucumerinum* because of the different symptoms it causes and the different optimum temperatures for disease development. *Fusarium solani* f. sp. *cucurbitae* is a cortical rot fungus causing symptoms similar to the new pathogen, but the two fungi are very distinct in microconidiophore morphology (3). In *F. oxysporum*, microconidia are borne on single phialides arising laterally on the hyphae or from short sparsely branched conidiophores. In *F. solani*, microconidia are formed from lateral con-

idiophores that initially may be merely elongated lateral phialides that narrow slightly toward the apex. Later formed microconidiophores are elongated and sparsely branched, reaching up to 400 µm long; each branch usually terminated in a single cylindrical to a barely subulate phialide that measures 45 to 80 × 2.5 to 3 µm. These are in marked contrast to the short microconidiophores with numerous phialides formed in *F. oxysporum*.

The pathogen causing root and stem rot of greenhouse cucumber in Crete is undoubtedly *Fusarium oxysporum* Schlechtend.: Fr., as interpreted by Booth (3) and Gordon (4). However, it is clearly different from known formae speciales of *F. oxysporum* because of its pathogenicity to *C. sativus* under natural conditions in the greenhouse and to *C. sativus*, *C. melo*, and *L. aegyptiaca* under artificial inoculation. It is also distinct in the symptoms it causes. *Fusarium oxysporum* f. sp. *luffae* infects *C. melo* and *L. aegyptiaca* and the symptoms include decay at the soil line, internal browning, and dropping of leaves, which resemble the symptoms caused by the new pathogen (1). However, the new pathogen is different from *F. oxysporum* f. sp. *luffae*, since the latter is not pathogenic on *C. sativus* (1). Because of this, a subspecific forma specialis designation should be used for isolates of *F. oxysporum* causing root and stem rot of *C. sativus*. Because the fungus is specific, or very nearly so, to *C. sativus*, it seems sensible to retain an epithet that denotes this, but it is also necessary to recognize the difference between this pathogen and *F. oxysporum* f. sp. *cucumerinum*. Accordingly, I propose to designate the fungus *Fusarium oxysporum* f. sp. *radicis-cucumerinum* f. sp. nov., following the precedent of both Weimer (11), who named the fungus causing a foot rot of *Lupinus* spp. as *F. oxysporum* f. sp. *radicis-lupini* J. L. Weimer, to differentiate it from a previously named wilt-causing fungus *F. oxysporum* f. sp. *lupini*, and Jarvis and Shoemaker (5), who named the fungus causing a foot and root rot of *Lycopersicon* spp. as *F. oxysporum* f. sp. *radicis-lycopersici* W. R. Jarvis & Shoemaker, to differentiate it from the wilt-causing fungus *F. oxysporum* f. sp. *lycopersici* (Sacc.) W. C. Snyder & H. N. Hans. A culture of *F. oxysporum* f. sp. *radicis-cucumerinum* (isolate AF-1(a)) has been deposited at the Commonwealth Agricultural Bureau International Mycological Institute (Egham, UK) (IMI 339891).

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