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FUSARIUM JAVANICUM KOORDERS IN ISRAEL

by

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ABSTRACT

Fusarium javanicum KDS. has been isolated from wilted vegetable marrows and melons and from the soil of a citrus grove in Israel. The morphology of the species is described, and measurements and drawings of macroconidia are presented. *In vitro* tests showed that *F. javanicum* failed to develop at either 12 or 40° C, and developed better at 24°C than 18 or 30°C. Mycelial growth on potato dextrose agar was always better at pH 7 than at pH 4.2. Darkness favored such growth to a marked extent only at 18° C and pH 4.2. In glasshouse inoculation tests one isolate of *F. javanicum* from vegetable marrow proved clearly pathogenic to cucumber, melon, watermelon and marrow. Measurements of macroconidia have been made, for comparative purposes, on isolates of *F. javanicum* var. *ensiforme* and of *F. solani* f. *cucurbitae* furnished from abroad, and on isolates of *F. solani* from cucurbits in Israel. Comparing the data for 3-septate macroconidia with those given in literature for *F. solani* and for *F. javanicum* and its var. *radicicola* and var. *ensiforme*, it is concluded that two groups can be distinguished: One group comprises, with one exception, all isolates of *F. javanicum* and its varieties as well as *F. solani* f. *cucurbitae*, and is characterized by slender macroconidia. The other group is represented by *F. solani* with its typically sausage shaped conidia. It is concluded that *F. javanicum* should be maintained as a species distinct from *F. solani*. It is further suggested that what has been called *F. solani* f. *cucurbitae* may have closer affinity to *F. javanicum* than to *F. solani*.

INTRODUCTION

In the course of a comprehensive study of the Fusaria of Israel, a Fusarium belonging to the section *Martiella*, but distinct from *F. solani* (MART.) APP. et WR., has on several occasions been isolated from wilting cucurbits and from soil. The species has been identified as *F. javanicum* KOORDERS and has been studied as to its morphology, environmental relationships *in vitro* and pathogenicity to cucurbits. For comparative purposes, the study comprised, especially in its taxonomic part, isolates supplied from abroad, including *F. javanicum* var. *ensiforme* from an unknown host, and various isolates of *F. solani* f. *cucurbitae*, as well as local isolates of *F. solani* from cucurbits.

In the following part a description will be given of the results of these studies and of the taxonomic considerations justifying the maintenance of *F. javanicum* as a species separate from *F. solani*.

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MATERIALS AND METHODS

Source of isolates

The isolates from cucurbits came from severely wilted fields of local cultivars of vegetable marrows (*Cucurbita pepo*) and melons (*Cucumis melo*) in the central part of the Valley of Yezreel, where the soil is heavy. Five isolates were made from 3 melon fields, and 4 from 2 marrow fields. From two of the melons, and one of the marrows, samples *F. solani* was isolated in addition to *F. javanicum*, and one melon sample yielded *F. equiseti* and another *F. equiseti* and *F. oxysporum* in addition to *F. javanicum* (JOFFE & PALTI, 1967).

The isolates kindly sent to us from abroad included: one of *F. solani* f. *cucurbitae* from Italy, made available by DR. C. M. MESSIAEN, Station de Pathologie Végétale, Montfavet, France; one isolate of *F. solani* f. *cucurbitae* race 1 from squash from Australia, and one isolate of race 2 of the same fungus from squash from Washington State, U.S.A., both sent by Prof. W. C. SNYDER, University of California, Berkeley, California; and 1 isolate of *F. javanicum* var. *ensiforme* from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.

The single soil isolate of *F. javanicum* was derived from light soil of a citrus grove in Israel's central coastal plain (JOFFE, 1967).

Isolation and culturing procedures

Isolates from wilted cucurbits were made by disinfecting small portions of their roots with 1:1000 mercuric chloride, rinsing 4 times in sterile water, and plating on potato dextrose agar at pH 4.0—4.2. During an 11-day period, each part of the root from which Fusarium developed was then transferred to slants.

If the slants remained free from contamination, they were checked for macroconidia. If these had not formed, transfers were made to two substrates (JOFFE, 1963, a, b): (1) BILAI's (1955) medium, slightly modified, consisting of 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 \cdot 7\ H_2O$, 0.5 g KCl, 0.2 g soluble starch powder, glucose 0.2 g, sucrose 0.2 g and water to make up 1000 cc. Five grams of the above were introduced into each test-tube together with small strips of pure cellulose paper. (2) Portions of *Melilotus alba* stalks sterilized in water. Macroconidia always formed on these substrates.

If more than one species of Fusarium was present, as was frequently the case, single spore cultures were made. Spore measurements were made on two slides from each isolate, with at least 100 macroconidia measured. Only measurements of 3-septate conidia, were used for comparative purposes.

Inoculation

Cucurbit plants were grown in glasshouses in small pots, in unsterilized soil. They were inoculated, mostly just after formation of the

TABLE I. Measurements of macroconidia of isolates of *F. javanicum* made in Israel

No. of isolate	3-septate conidia			4-septate conidia			5-septate conidia		
	%	measurements		%	measurements		%	measurements	
		frequent	extreme		frequent	extreme		frequent	extreme
1. *Cucumis melo*									
214*	24	42—51 × 4.0—4.4	34—56 × 3.7—4.6	41	44—54 × 4.2—4.3	37—59 × 3.8—4.5			
285	31	35—46 × 4.5—4.8	27—50 × 4.2—5.2	8	42—51 × 4.6—5.0	34—55 × 4.3—5.4	23	45—54 × 4.4—4.9	36—59 × 4.1—5.3
301	16	44—50 × 4.4—4.7	37.0—54 × 4.0—5.0	39	46—52 × 4.4—4.8	42—56 × 4.0—5.1	32	49—60 × 4.4—5.0	43—65 × 4.2—5.3
308	5	36—44 × 4.2—4.5	28—51 × 3.8—4.7	14	41—51 × 4.4—4.6	36—55 × 4.0—4.9	44	45—49 × 4.2—5.0	38—63 × 4.0—5.5
2. *Cucurbita pepo*									
190	8	36—47 × 4.3—5.0	29—51 × 4.0—5.3	37	43—50 × 4.4—5.2	33—53 × 4.1—5.4	19	45—56 × 4.3—5.2	36—61 × 4.0—5.5
199	6	39—48 × 4.2—4.9	28—52 × 3.6—5.2	22	47—54 × 4.2—5.0	36—59 × 3.6—5.2	59	51—62 × 3.9—5.2	42—69 × 3.5—5.4
206	41	36—43 × 4.0—5.0	24—47 × 3.5—5.2	30	42—49 × 4.4—5.2	35—53 × 3.6—5.4	21	41—55 × 4.6—5.4	38—59 × 4.0—5.5
300	7	38—44 × 4.7—5.0	34—51 × 4.3—5.4	41	42—56 × 4.5—5.2	35—65 × 4.2—5.5	17	49—61 × 4.2—4.8	41—71 × 4.0—5.0
3. *Citrus soil*									
3585	43	34—45 × 3.9—4.5	28—51 × 3.5—4.9	17	41—51 × 4.0—4.6	36—55 × 3.4—4.8	21	46—53 × 4.1—5.0	39—59 × 3.9—5.2

* isolate 214 also contained 3 % of 6-septate conidia

first true leaves, by application of mycelium and agar to 2 cm deep furrows scooped out by finger next to the plants. Each test series comprised 40—50 plants of each host (except series 21 in which only 20 plants were used). Details of this inoculation method have been described elsewhere (PALTI & JOFFE, in press).

RESULTS

Morphology of the Israeli Isolates of F. javanicum

The nine isolates of *F. javanicum* from cucurbits and the one from citrus soil all made whitish, or slightly yellowish, powdery growth on potato dextrose agar, and in general formed macroconidia on this substrate quite readily.

As seen from Table I, these isolates contained conidia with 3, 4 or 5 septae in widely varying proportions, but only one isolate had conidia with more than 5 septae. The percentage of 3-septate conidia never exceeded 43.

Conidia were always slender, not exceeding 5.5 μ in width even in extreme measurements, and with a minimum width of 3.5—3.8 μ.

In vitro effects of temperature, light and substrate

One isolate of *F. javanicum* and one of *F. solani*, both derived from the same vegetable marrow plants, were grown in culture on potato dextrose agar adjusted to pH 4.2 and 7 in thermostats at temperatures of 12, 18, 24, and 32, and at 40$\pm$1° C. The thermostats were illuminated with fluorescent light of the 'Warmwhite' type, at 20 watt intensity. Half the number of the plates were wrapped in silver paper, which was not opened until the end of the test, so that the fungus in those plates grew in complete darkness.

TABLE II. Effects of temperatures, light and pH of substrate on growth of *F. javanicum* and *F. solani* in culture

Temp. ° C	Light conditions	Growth (in mm) made after 7 days' culturing (mean of 4 plates)			
	pH of substrate:	*F. javanicum*		*F. solani*	
		4.2	7.0	4.2	7.0
12	light	0	0	18	15
	dark	0	0	2	4
18	light	6	41	51	88
	dark	26	49	44	89
24	light	45	72	50	85
	dark	47	74	55	73
32	light	40	57	66	79
	dark	34	66	70	89
40	light	0	0	0	0
	dark	0	0	0	0

There were four plates to each treatment. Results were determined by measuring the diameter of the colony that had formed after 7 days.

As seen from Table II, the following differences between the two isolates were in evidence: (1) At 12° C *F. javanicum* failed to grow on either substrate, either in light or darkness, where as *F. solani* grew fairly well in light. (2) At 18° C, the growth made by *F. solani* at each possible combination of light and substrate, was much better than that made by *F. javanicum.* (3) Both species grew better at pH 7 than at pH 4.2. (4) At temperatures favorable to growth (18, 24, 30° C) the effect of light and darkness was not prominent. (5) Neither of the isolates grew at 40° C.

Pathogenicity tests

In a preliminary test, pathogenicity of the local *F. javanicum* isolate 199/10 from vegetable marrows was compared with that of the Dutch isolate designated as *F. javanicum* var. *ensiforme.* The former caused 79 % wilt, the latter 33 %.

Results obtained in 5 test series with the same isolate inoculated on cucumber, melon, watermelon and marrow, are summarized in Table III. Results refer to the percentage of plants wilted 30—40 days after inoculation. The isolate was clearly pathogenic to all four of these cucurbits. A comparison of the pathogenicity to cucurbits of Israeli isolates of *F. javanicum, F. solani, F. oxysporum* and *F. equiseti* has been presented by PALTI & JOFFE (in press).

TABLE III. Pathogenicity of an isolate of *Fusarium javanicum* from vegetable marrows to various cucurbits

Test series	host age at inoculation (days)	percentage of plants wilted							
		cucumbers		melons		watermelons		marrows	
		inoculated	control	inoculated	control	inoculated	control	inoculated	control
15	0*	38	10	68	0	96	0	100	7
16	10			95	3	98	3	100	0
18	14	74	6	52	0	100	26	78	0
21	36			89-100**	0	80-96***	0		
22	18			70-100**	0-10	57-82	0		

* inoculated at the time of sowing
** mean of 8 melon varieties
*** mean of 3 watermelon varieties

Taxonomy of F. javanicum

The proposal by SNYDER & HANSEN (1941) to include all species of the section *Martiella* in the collective species *F. solani* has been widely accepted by phytopathologists, if not by mycologists.

TABLE IV. Measurements of 3-septate conidia of isolates of *F. solani*, *F. javanicum* and some of their varieties as determined in studies in Israel and abroad

Reference	Source	measurements (in)	
		frequent	extreme
1. *Fusarium javanicum* KOORD.			
WOLLENWEBER & REINKING (1935)		28—47 × 4—5	22—54 × 3.5—6.0
BUGNICOURT (1939)	4 hosts (mean)	36 × 4.6	24—47 × 3.7—5.4
JOFFE & PALTI	Cucumis melo (mean of 4 isol.)	39.3—47.8 × 4.3—4.6	31.5—52.8 × 3.9—4.9
	Cucurbita pepo (mean of 4 isol.)	37.3—45.5 × 4.3—5.0	28.8—50.3 × 3.9—5.3
JOFFE (1967)	citrus soil	34—45 × 3.9—4.5	28—51 × 3.5—4.9
2. *Fusarium javanicum* KOORD. var. *ensiforme* (WR. & RG.) RAILLO			
RAILLO (1950)		44 × 3.4	37—50 × 3.7—5.0
JOFFE & PALTI	unknown host (Dutch isolate)	48—55 × 4.4—4.8	41.5—62 × 4.1—5.0
3. *Fusarium javanicum* KOORD. var. *radicicola* WR.			
WOLLENWEBER & REINKING (1935)		30—42 × 3.8—4.8	20—50 × 3.4—5.3
RAILLO (1950)		30—38 × 3.7—4.6	20—44 × 3—4.5
BUGNICOURT (1939)	Hevea brasiliensis	33 × 4.3	27—38 × 3.6—4.8
	Desmodium ovalifolium	34 × 4.1	23—43 × 2.9—5.1
4. *Fusarium solani* (MART) APP. & WR. f. *cucurbitae* SNYD. & HANS.			
JOFFE & PALTI	Cucurbita pepo (from Australia)	25.5—37 × 3.3—4.4	20.8—40.5 × 3.1—4.9
	Cucurbita pepo (from Washington State, USA)	22—31 × 3.3—4.5	18—33.5 × 2.6—5.2
	unknown host (from Italy)	26—31 × 2.7—4.5	21—33.5 × 2.5—5.2
5. *Fusarium solani* (MART.) APP. & WR.			
WOLLENWEBER & REINKING (1935)		28—42 × 4.1—6.2	19—50 × 3.5—7.0
RAILLO (1950)		31.9—40.2 × 5.5—6.0	21—47 × 4.0—7.0
BILAI (1955)		30—45 × 4.5—5.5	20—60 × 4.0—7.0
GORDON (1952)		27.6—33.9 × 4.5—5.9	
JOFFE & PALTI	Cucumis sativus (mean of 31 isol.)	30.4—42.9 × 4.7—6.4	24.4—49.2 × 4.4—6.7
	Cucumis melo (mean of 13 isol.)	28.8—38.5 × 5.0—6.2	23.7—45.1 × 4.8—6.5
	Citrullus vulgaris (mean of 6 isolates)	31.2—41.8 × 5.1—6.7	25.0—5.0 × 4.8—6.9
	Cucurbita pepo (isolate)	28—40 × 4.4—6.6	22—46 × 4.2—7.0

The morphological comparison presented in Table IV has as its purpose to arrive at a reasoned conclusion, whether *F. javanicum* should really be included in *F. solani*. To this end, we present measurements of 3-septate conidia, as taken for the Israeli isolates of *F. javanicum* and as described by WOLLENWEBER & REINKING (1935), RAILLO (1950), and BUGNICOURT (1939) for *F. javanicum* and its var. *radicicola*, as well as RAILLO's (1950) measurements of *F. javanicum* var. *ensiforme*, and our measurements of the Dutch isolate of this variety. These measurements are being compared with

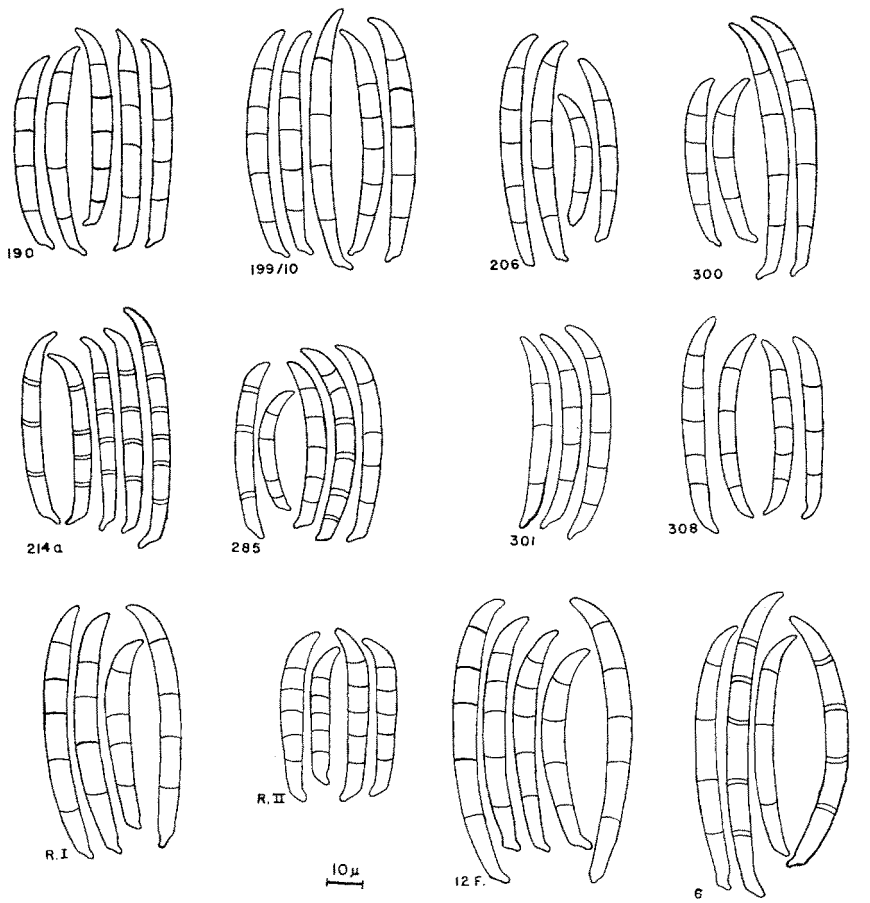

Fig. 1. Macroconidia of *Fusarium javanicum* and *F. solani* f. *cucurbitae*. Top row: *F. javanicum* isolated from vegetable marrow in Israel. Second row: *F. javanicum* isolated from melon in Israel. Third row: R I – *F. solani* f. *cucurbitae*, race 1, isolated from squash in Australia. R II – *F. solani* f. *cucurbitae*, race 2, isolated from squash in Washington State, U.S.A. 12 F – *F. solani* f. *cucurbitae* from Italy. 6 – *F. javanicum* var. *ensiforme*.

those published for *F. solani* by WOLLENWEBER & REINKING (1935), RAILLO (1950), BILAI (1955), and GORDON (1952) with our measurements of 51 isolates of *F. solani* from 4 cucurbit hosts in Israel, and with our measurements of the 3 isolates of *F. solani* f. *cucurbitae* sent to us from abroad.

According to these measurements, and especially the width of the 3-septate conidia, the fungi referred to in the table may be divided into 2 distinct groups: one group includes *F. javanicum* with its two varieties, as well as *F. solani* f. *cucurbitae*. In this group frequent measurements for maximum width of conidia invariably ranged from 3.4 to 5.0 μ, and their minimum width from 2.7 to 4.4 μ, and the corresponding extreme measurements were, with one exception, 4.5 to 5.3 μ for maximum, and 2.5 to 3.9 μ for minimum width. The Dutch isolate of *F. javanicum* var. *ensiforme*, as measured in Israel,

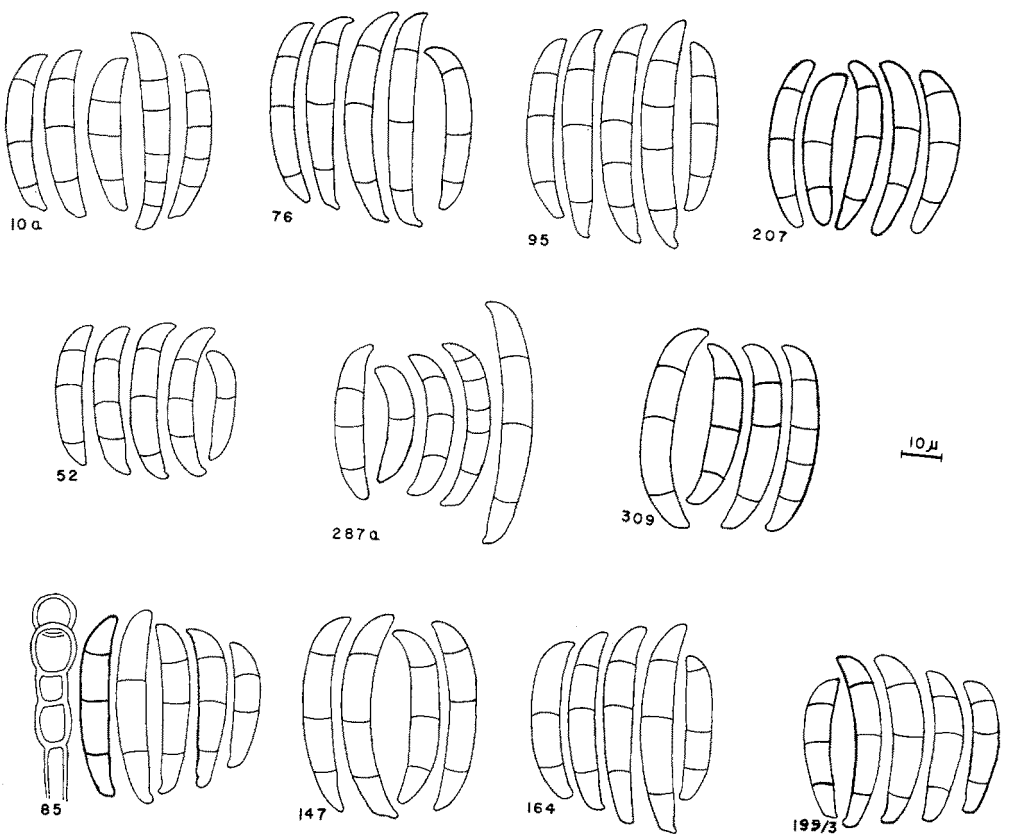

Fig. 2. Macroconidia of *Fusarium solani* isolated from cucurbits in Israel. Top row: isolates from cucumber. Second row: isolates from melon. Third row: Nos. 85, 147, 164 – isolates from watermelon. No. 199/3 – isolate from vegetable marrow.

deviated from the others in this group in several respects and probably does not belong into the same group.

The second group is constituted by the isolates of *F. solani*. Here frequent measurements for maximum width of conidia ranged from 5.5 to 6.7 μ, and for their minimum width from 4.5 to 5.5 μ; the corresponding extreme measurements were 6.3 to 7.0 μ for maximum and 4.0 to 5.2 μ for minimum width. The sole exceptions, as regards both frequent and extreme measurements for minimum width, were those of WOLLENWEBER & REINKING (1935).

Repeated transfers of the Israeli isolates of *F. javanicum* and *F. solani*, and of the *F. solani* f. *cucurbitae* isolates sent from abroad, did not alter the above differences between the two groups. These differences are also clearly shown in Figs. 1 and 2.

Isolates exhibiting a difference in conidial width constantly exceeding 1 μ and often reaching 2 μ, should not, in our view, be considered as belonging to the same taxonomic unit.

Another difference between the Israeli isolates of *F. javanicum* and *F. solani* from cucurbits consisted in the respective prevalence of 3-septate conidia. As mentioned above, the proportion of these conidia in *F. javanicum* did not exceed 43 % and was mostly much lower. But in the 51 isolates of *F. solani*, 3-septate conidia were far more prevalent, and usually made up 60—90 % of all conidia. While this in itself is not reliable enough a criterion, it may be considered as an auxiliary means of distinguishing between the species.

In sum, we propose to maintain *F. javanicum* KOORDERS with its, slender, elongated macroconidia, as a species distinct from *F. solani* (MART.) APP. & WR., with its typically sausage shaped conidia.

As mentioned above, SNYDER & HANSEN (1941) have included *F. javanicum* in the collective species *F. solani*. Our study of isolates from cucurbits sent to us with the designation *F. solani* f. *cucurbitae* leads us to the conclusion that the isolates may have closer affinity to *F. javanicum* than to *F. solani*.

Zusammenfassung

Fusarium javanicum KOORDERS wurde in Israel von verwelkten Cucurbitaceen *(Cucurbita pepo* 'Kussa' and *Cucumis melo)* sowie von dem Boden einer Citruspflanzung isoliert. Die Morphologie des Pilzes wird hier beschrieben. In *in vitro* Versuchen entwickelte sich *F. javanicum* auf Kartoffel-Dextrose Agar weder bei 12 noch bei 40° C, und wuchs besser bei 24 als bei 18 oder 30° C. Das Wachstum des Myzeliums war stets besser bei pH 7 als bei pH 4.2. Dunkelheit förderte dieses Wachstum erheblich nur bei 18° C und pH 4.2. In Impfungsversuchen im Glasshaus wirkte ein Isolat des *F. javanicum* von *C. pepo* stark pathogenisch auf Gurken, Melonen, Wassermelonen und *C. pepo*. Zwecks taxonomischer Vergleiche wurden Makrokonidien aus dem Ausland erhaltener Isolate von *F. javanicum* var. *ensiforme* und *F. solani* f. *cucurbitae* gemessen, sowie solche des *F. solani* von Cucurbitaceen in Israel. Die Resultate dieser Messungen wurden mit den in der Literatur angegebenen Werten für *F. solani*, *F. javanicum* und dessen var. *radicicola* und var. *ensiforme* verglichen. Dieser Vergleich zeigte 2 klar getrennte Gruppen: Die erste

enthält, mit einer Ausnahme, alle Isolate des *F. javanicum* und des *F. solani* f. *cucurbitae*, und besitzt typisch schlanke Makrokonidien. Die zweite Gruppe besteht aus *F. solani* mit seinen typisch dickeren, Würstchenartigen Sporen. Dies führt zu dem Schluß, daß *F. javanicum* weiterhin als eine von *F. solani* getrennte Spezies zu betrachten ist.

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