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Pathogenicity of Fusarium solani f.sp. cucurbitae race 1 to courgette

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Abstract

Fusarium solani f. sp. *cucurbitae* race 1 causes foot rot in courgette (*Cucurbita pepo*). The pathogen could be distinguished from *Fusarium solani* from sweet pepper (*Capsicum annuum*) both morphologically and in its host range.

In inoculation experiments all nine cultivars of the six species of Cucurbitaceae tested were susceptible. Courgette 'Green' became diseased after inoculation with a spore suspension by root dipping or adding the suspension to the soil around the stem base or spraying the whole plant with it.

Both wounded and young plants died more quickly than unwounded and older plants. With low inoculum densities the plants were affected more slowly than with high densities and the differences in susceptibility of the Cucurbitaceae tested were more pronounced.

From infected courgette seeds the fungus could be reisolated until 6 months after harvest.

This is the first record of this pathogen in courgettes in the Netherlands.

Additional keywords: Cucurbita pepo, seed infection.

Introduction

In the Netherlands, courgette (*Cucurbita pepo*), is grown under glass since the middle of the 1970s. The acreage is about 9 ha. Since 1980 a severe foot rot occurs in this crop in several nurseries. In spite of soil disinfestation by means of steam or methyl bromide, 25 per cent of plants can be dead at the end of the cropping period. In crops grown on rockwool foot rot occurs only occasionally.

The first symptoms appear a few weeks after planting. The plants become dark green and are retarded in their growth. The stem base is soft and pulpy and many roots are affected. Lesions may be found higher on the stem but then an existing wound acted as site of infection. On the lesion sometimes a grey mass of *Fusarium*-like macroconidia is visible. Plants with these symptoms die within a few weeks, and this occurs during the whole growing season. The sexual stage of that fungus has never been observed on plants.

The fungus was isolated from diseased plants at the Glasshouse Crops Research Station and the Plant Protection Service and identified as *Fusarium solani* (Mart.) Sacc. f. sp. *cucurbitae*, the conidial stage of *Nectria haematococca* Berk. & Br. var. *cucurbitae*. Two races of *F. solani* f. sp. *cucurbitae* are known. Race 1 attacks roots, stems and fruits, race 2 only the fruits (Toussoun and Snyder, 1961). This means that

in the Netherlands race 1 of this pathogen occurs.

In the South-Holland glasshouse district, the pathogen has been isolated from cucumber in 1958, 1962 and 1963. It was probably introduced by means of infected seed of *Cucurbita ficifolia* Bouché, the rootstock for cucumber (Kerling and Bravenboer, 1967). By adequate soil disinfestation and the use of healthy seed the disease has disappeared from the South-Holland glasshouse district.

From sweet pepper (*Capsicum annuum*), *Fusarium solani* is often isolated from rotten roots, rotten stem bases, black coloured stems and fruits since 1974. On the rotten stem bases of diseased plants and on rotten fruits perithecia of *Nectria haematococca* can be found (Van Steekelenburg and Paternotte, 1979).

In the present study, growth characteristics of cultures of *F. solani* from sweet pepper and *F. solani* f. sp. *cucurbitae* from courgette have been compared and the pathogenicity has been tested.

Differences in susceptibility to *F. solani* f. sp. *cucurbitae* between and within various species of Cucurbitaceae, including *Cucurbita pepo*, are reported by Toussoun and Snyder (1961) and Vannacci and Gambogi (1980).

On Dutch holdings where c. 20% of ungrafted courgette plants died prematurely, plants grafted on the rootstock R.S. 841 or on *Benincasa hispida* (*cerifera*) (Thumb.) cogn. remained healthy. Plants grafted on *B. hispida* (*cerifera*) had a slightly darker colour than those grafted on R.S. 841. R.S. 841 is a breeding-line of the seed company Royal Sluis. On the rootstock *B. cerifera* itself *F. solani* f. sp. *cucurbitae* was observed occasionally. In a heated crop of the courgette cv. Gold Rush planted in January, the plants were less severely infected than those of the courgette cv. Green. This was not observed in a summer-planted crop.

In the present study, cultivars of courgette and other Cucurbitaceae were tested in order to find out whether a less susceptible courgette cultivar is available or a less susceptible species which can be used as a rootstock.

The pathogen is transmitted very easily with seed and in this way it is spread to other holdings (Doidge and Kresfelder, 1932; Snijder, 1938: Conroy, 1953; Snyder and Hansen, 1954; Richardson, 1979). The seed can be infected both internally and externally. There is no consensus among authors about the period the fungus can survive in the seed (Gries, 1946; Toussoun and Snyder, 1961). Therefore, the duration of infectivity of infested seed was investigated.

Furthermore, the effect of removing dying leaves, of wounding the plant and of the plant age on the infection was studied.

Materials and methods

Culturing. Isolates of *F. solani* from sweet pepper and isolates of *F. solani* f. sp. *cucurbitae* race 1 from courgette were grown on Czapek Dox agar in an incubator at 25 °C. For morphological studies and in order to induce the perfect stage isolates were grown under black light at 20 °C in the laboratory and in a glasshouse at 27 °C. The isolates of *F. solani* f. sp. *cucurbitae* were cross-bred by placing two discs of 5 mm in diameter of cultures of different origin onto one plate. Growth response to temperature was assessed by measuring two perpendicular diameters of colonies originating from 5 mm discs from full-grown plates, after incubation for 6 d at different temperatures.

Conidial suspensions were obtained by culturing the fungus 5 to 7 days in liquid Czapek Dox at 26 °C.

Inoculation. Plants were inoculated by dipping the roots in a conidial suspension (10^6 spores ml^{-1}), or by adding the suspension to the stem base or by spraying the whole plant with it. In two experiments plants were wounded by cutting the first leaf from the stem and making a superficial radial incision in the stem lengthwise over 1 cm. In one experiment the conidial suspension was applied to the soil around the stem base and brushed onto the stem. In this experiment the lower yellow and dead leaf-blades of the plants were removed weekly, to create a drier microclimate around the stems.

The temperature in the glasshouse experiments was 23 °C or higher, depending on the global radiation inside the glasshouse.

Seed infection. Infected seeds of courgette were obtained by injecting a full-grown fruit of cv. Green at five places with 10 ml conidial suspension (10^6 spores ml^{-1}). After one month, when the fruit was totally rotten, the seeds were removed, rinsed with water and dried. The seeds were stored at room temperature.

Reisolations were made after disinfecting the seed externally with 0.1% sodium hypochlorite or 20 and 50% methanol.

After 7 and 10 months the percentage germination was assessed by sowing 300 and 100 seeds, respectively.

Results

Morphology. Isolates of *F. solani* f. sp. *cucurbitae* from courgette (n = 5) cultured on Czapek Dox agar were sometimes white or cream but they mostly showed a grey, brown-violet or blue-green pigmentation. The pigmentation depended on light intensity during incubation and age of the cultures. Isolates of *F. solani* from sweet pepper (n = 5) on Czapek Dox agar were in all circumstances white or cream with sometimes a blue pigmentation mostly in particular sections of the colony.

The perfect stage of *F. solani* f. sp. *cucurbitae* from courgette was never observed, neither in vitro nor in vivo. In contrast, the perfect stage of *F. solani* from sweet pepper was formed often on diseased sweet pepper plants and incidentally on agar plates when incubated in the light. On agar plates, *F. solani* f. sp. *cucurbitae* from courgette formed less aerial mycelium and sporulated more profusely than *F. solani* from sweet pepper.

The conidia of *F. solani* f. sp. *cucurbitae* were up to 65 μm long with 0 tot 5 septa and more slender than those of *F. solani* from sweet pepper which were up to 37.5 μm long with 0 to 3 septa.

Effect of temperature on mycelial growth. Five isolates of *F. solani* and *F. solani* f. sp. *cucurbitae* were grown at different temperatures on Czapek Dox agar. The minimum temperature of *F. solani* was lower and the maximum temperature higher than the comparable values for *F. solani* f. sp. *cucurbitae*. The colony diameter of *F. solani* always exceeded that of *F. solani* f. sp. *cucurbitae*. The colony diameters are given in Fig. 1.

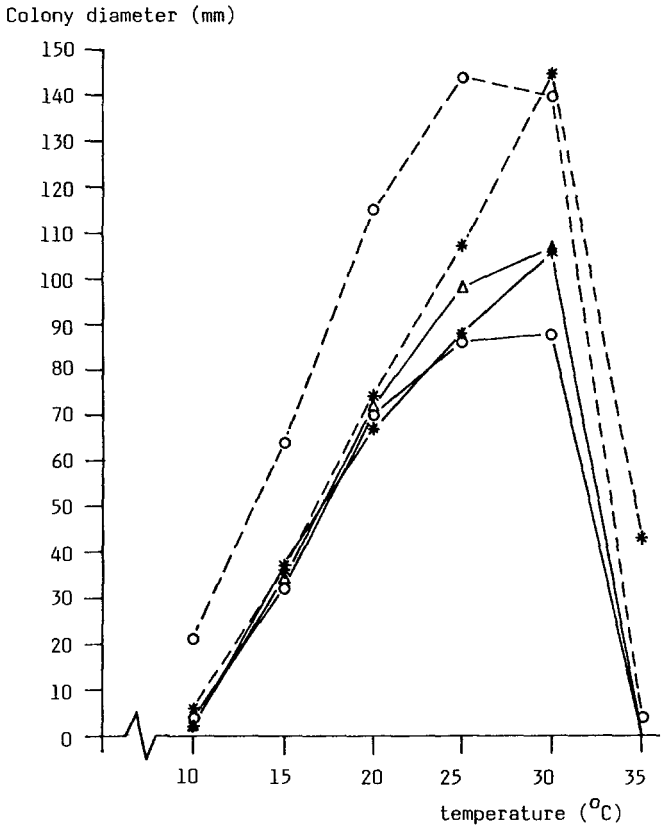

Fig. 1. Colony diameter in mm of isolates of *F. solani* f. sp. *cucurbitae* from courgette (———/○ * △) and *F. solani* from sweet pepper (---------/○ *) after growth for 6 days at different temperatures on Czapek Dox agar (mean of 3 replicates).

Host range. Isolates from courgette affected only Cucurbitaceae and did not affect sweet pepper and eggplant. Isolates from sweet pepper only affected sweet pepper. This infection was slight and only successful after wounding. The other crops tested were not affected (Table 1).

Inoculation methods. All Cucurbitaceae mentioned in Table 1 which were inoculated by dipping the roots of seedlings in a conidial suspension of *F. solani* f. sp. *cucurbitae* showed symptoms within one week and died for the greater part within 2 weeks. One-month-old wounded courgette plants which were sprayed with a conidial suspension or to which 35 ml of the suspension was applied around the stem base were diseased equally fastly and seriously.

Unwounded plants of the same age, which were sprayed with a conidial suspension, showed a slight brown discoloration on the stem base. Three-months-old unwounded plants to which a conidial suspension was applied around the stem base, were dead

Table 1. Plant species tested for susceptibility to isolates of *F. solani* from sweet pepper and *F. solani* f. sp. *cucurbitae* from courgette.

Test plant		*F. solani*	*F. solani* f. sp. *cucurbitae*
Cucurbita pepo 'Green'	(courgette)	−[2]	+[2]
Cucurbita pepo 'Gold Rush'	(yellow courgette)	n.t.[2]	+
Cucurbita pepo 'Pattison'		n.t.	+
Citrullus vulgaris 'Sweet Baby'	(watermelon)	n.t.	+
Citrullus vulgaris 'Gold Baby'	(watermelon)	n.t.	+
Cucurbita ficifolia		−	+
Benincasa hispida(cerifera)		n.t.	+
Cucumis sativus 'Farbio'	(cucumber)	−	+
Cucumis melo 'Ha'on'	(melon)	−	+
'R.S. 841'[1]		n.t.	+
Capsicum annuum 'Tisana'	(sweet pepper)	+	−
Solanum melongena 'Mammouth'	(eggplant)	−	−

[1] Breeding-line of Royal Sluis.
[2] + or − : diseased or not diseased after inoculation; n.t.: not tested.

after one month. When the suspension was brushed onto the stem from which all yellow and dead leaves had been removed, only 1 out of 7 plants was dead after one month, whereas 5 out 7 plants were dead when leaves had been left.

Plant age. Wounded courgette plants of 2- till 8-weeks old to which a conidial suspension of *F. solani* f. sp. *cucurbitae* was applied around the stem base were all diseased after 12 days, but speed of death reduced with the age of the plants at inoculation; young plants (up till 4 weeks old) were all dead within 12 days after inoculation and 6- and 8-weeks-old plants were nearly all dead after 26 days.

Table 2. Numbers of diseased and dead courgette plants cv. Green after inoculation with a spore suspension of *F. solani* f. sp. *cucurbitae* in different inoculum densities by root dipping or adding the suspension to the soil around the stem base of seedlings (20 plants per treatment).

Inoculum density (conidia per ml)	Days after inoculation							
	15		23		33		42	
	diseased	dead	diseased	dead	diseased	dead	diseased	dead
10^2	0	0	3	0	7	6	3	12
10^3	0	0	4	0	4	12	5	15
10^4	6	0	14	2	4	16	3	17
10^5	14	3	11	6	0	20	0	20
10^6	12	8	12	8	3	17	2	18

Table 3. Numbers of diseased and dead plants of species of Cucurbitaceae 28 days after dipping the roots in a conidial suspension of *F. solani* f. sp. *cucurbitae* with different amount of spores (48 plants per treatment).

Test plant	Number of spores per ml suspension									
	10^2		10^3		10^4		10^5		10^6	
	diseased	dead	diseased	dead	diseased	dead	diseased	dead	diseased	dead
C. pepo 'Green'	4	44	0	48	0	48	0	48	0	48
C. pepo 'Gold Rush'	3	45	0	48	0	48	0	48	0	48
C. sativus 'Farbio'	3	0	7	3	12	18	12	28	0	48
B. hispida (cerifera)	6	9	3	43	0	48	0	48	0	48
R.S. 841	—	—	18	18	9	39	1	47	0	48

– not included.

Inoculum density. Low inoculum densities caused a less rapid infection to Cucurbitaceae than high inoculum densities (Table 2). Moreover differences in susceptibility for the pathogen between the species of Cucurbitaceae tested were more pronounced (Table 3). Processing the data by analysis of variance revealed a significant interaction between inoculum density and disease incidence.

Seed infection. Twenty percent of the courgette seeds harvested from the infected fruit showed discoloration from light brown to black. The pathogen was reisolated after disinfecting the seed externally until 6 months after collecting, but not after a longer storage period. The percentage of seeds that germinated 7 and 10 months after collecting was 50 and 67, respectively. Seeds from a non-inoculated fruit, stored under the same conditions, germinated for 95% both after 7 and 10 months. Thirty percent of the young seedlings of the infected lot had necrotic lesions on the cotyledons. However, it was not possible to isolate the pathogen from these lesions.

Discussion

Isolates of *F. solani* f. sp. *cucurbitae* from courgette can macro- and microscopically be distinguished from *F. solani* from sweet pepper. The host range of the two pathogens also differed. This is the first record of this pathogen in courgettes in the Netherlands.

For different reasons it is unlikely that this pathogen originates from cucumber. The pathogen can survive in soil for only a few years (Nash and Alexander, 1965; Walker, 1952). The period between the last observation in cucumber in the South-Holland glasshouse district, more than 20 years ago, and the recent observation in courgette seems much too long for survival in soil, especially because on cucumber-holdings infested soils were heavily steam-sterilised. Moreover, the infestation of cucumber crops in the South-Holland area probably occurred exclusively through plant propagation nurseries by way of infected seeds of *C. ficifolia*, the rootstock for

cucumber (Kerling and Bravenboer, 1967).

With high inoculum densities of the pathogen none of the *Cucurbitaceae* tested was resistant. When lower inoculum densities were used differences in susceptibility to the pathogen, even between cultivars of *C. pepo*, became apparent. However, if other isolates are used the differences may vary again (Vannacci and Gambogi, 1980). The differences in susceptibility between the Cucurbitaceae found in the experiments are confirmed by the observation on holdings mentioned in the introduction with a severe incidence of *F. solani* f. sp. *cucurbitae*. More material of low susceptibility might exist within species of Cucurbitaceae.

Plants of less susceptible Cucurbitaceae can escape infection when inoculum densities in the soil are low, as has been reported for cucumber (Kerling and Bravenboer, 1967).

It is possible that the fungus has been introduced in courgette in the South-Holland glasshouse district by means of infected seed. Seed is imported from the USA. The seed of cultivar Green, one of the most commonly used cultivars here, was easily infected experimentally with the pathogen. The fungus could be reisolated without difficulty even from seeds that were externally disinfected. This suggests that the seed is infected internally. Spread of the pathogen to new areas by means of infected seed has been confirmed by Toussoun and Snyder (1961).

The pathogen was not viable anymore in and on the seed after a storage period of 7 months. This is in contradiction with results of Gries (1946) and Conroy (1953) who found that the fungus remained viable in seed for 2 years and longer. Toussoun and Snyder (1961) found that the viability of infected seed is not impaired, and its germinability remains generally unaffected, when seed was infected internally. In my investigations an obvious reduction in the percentage of emergence was found. This may have been caused by a difference in inoculation method and consequently in the extent of seed infection or by the circumstances under which the infected seed germinated (Vannacci and Gambogi, 1980).

The low degree of infection observed when all dying leaves had been removed before inoculation of the stem is probably due to the reduction of the relative humidity in the crop brought about by the deleaving treatment.

It was neither possible for older courgette plants cv. Green nor for wounded courgette plants to escape infection.

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Samenvatting

Voetrot van courgette, veroorzaakt door Fusarium solani f. sp. cucurbitae

Fusarium solani f. sp. cucurbitae fysio 1 is de oorzaak van een voetrot in courgette. Het pathogeen is morfologisch en door middel van een waardplantenreeks goed van *F. solani* uit paprika te onderscheiden.

In inoculatieproeven waren de getoetste Cucurbitaceae in meer of mindere mate gevoelig voor *F. solani* f. sp. *cucurbitae*. Bij courgette 'Green' werden zowel gedompelde, als aangegoten, als bespoten planten door het pathogeen aangetast. Zowel verwonde als niet-verwonde planten werden aangetast als ook planten van verschillende leeftijden. Niet-verwonde en ook oudere planten stierven minder snel af dan verwonde en jongere planten. Bij lagere inoculumdichtheden werden de planten minder snel aangetast dan bij hogere dichtheden en waren de verschillen in vatbaarheid voor het pathogeen tussen de getoetste Cucurbitaceae duidelijker.

Uit courgette zaad, dat met het pathogeen was besmet, kon de schimmel tot 6 maanden na zaadwinning opnieuw worden geïsoleerd.

Dit is de eerste melding van dit pathogeen in courgette in Nederland.

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