J. Agr. Sci. Tech. (2000) Vol. 2: 27-32

Symptomless Carriers of the Causal Agent of Tomato Wilt Pathogen

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ABSTRACT

A study was carried out to determine whether naturally-occurring weeds and other cultivated plants in tomato growing regions could act as symptomless reservoirs of infection to *Fusarium oxysporum f. sp. lycopersici.* A numher of *F. oxysporum* isolates from weeds and oubergines were used in this investigation. A susceptible tomato cultivar was used for comparison. The plants were artificially inoculated at the five to six leaf stage by root dip method. Only tomato showed wilt symptoms and died three weeks after inoculation. These isolates were identified as F. o. f. sp. lycopersici. Weeds including *Amaranthus retroflexus, Amaranthus.* sp., *Chenopodium album,* and aubergines were colonized to various degrees and determined as symptomless carriers. Therefore, in infested areas, aubergines should not be rotated consecutively with tomatoes and proper measures should be adopted to control the weeds.

Keywords: Lycopersicom esculenttim Mill , Symptomless carrier , *Fusarium oxysporum f. sp. lycopersici* .

INTRODUCTION

Vascular wilt *Fusaria* can survive in the absence of susceptible host plants by invasion and colonizaion of other plants that show no symptoms of the disease [1, 8, 17, 18]. These plants are often described in the literature as symptomless carriers [1,8], non-susceptible hosts [7,12], or non-hosts [3|. Katan [8] studied symptomless carriers of *Fusarium oxysporum* Schlecht. f. sp. lycopersici (Sacc.) Synd. and Hans (Fol). A number of weeds growing in soil naturally infected with the pathogen did not show wilt symptoms, although they harbored the pathogen. These weeds included the genera of *Oryzopsis, Digitaria, Amaranthus,* and *Malva*. The plants were also colonized when planted under controlled conditions in soil naturally infested with race 1 and 2 of the pathogen, or when artificially inoculated with isolates of the pathogen from roots of weeds or tomatoes *{Lycopersicom esculentum* Mill.)- In a more recent study, Benincasa (a wild cucurbit), also a non-host, showed no wilt symptoms following inoculation with *F. o. f. sp. melonis*. The fungus occurred in the root, but never in the stem [3].

In Hormozgan province, where *Fusarium* wilt of tomato can be a serious problem [5], aubergine *(Solarium melongena* L.) is widely used in rotation with tomato. In addition, several species of common weeds including *Amaranthus retroflexus, Amaranthus.* sp., and *Chenopodium album* occur in these crops. The following study was conducted to determine whether naturally occurring weeds and

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[DOR: 20.1001.1.16807073.2000.2.1.1.7]

aubergines could act as symptomless carriers of *Fol*

MATERIALS AND METHODS

Culture of the Host and Pathogen

Aubergine seeds cv. Long Purple were germinated in compost at 20-25°C in a glasshouse for two weeks. When emergence was complete, seedlings were pricked off into 12 cm pots, usually after the first true leaf had clcaity emerged. Seedlings were selected for uniformity and deformed ones discarded. At the two leaf stage, seedlings were transferred to 12 cm pots and kept for a period of 5-6 weeks until the sixth leaf stage when these were suitable for inoculation. From the fourth week onwards, the plants were supplied with 50 ml Hoagland solution on alternate days. Seeds of weeds, *A. retroflexus, A. sp.* and *C. album* were also sown in compost.

Isolates of *F. oxysporutn,* obtained from aubergines and weeds, were used in the present study. These isolates were identified as *Fol* race 1 using a method similar to that previously described [6]. Conidial suspensions of *Fol* isolates were prepared by adding 10 ml aliquots of sterilized distilled water (SDW) to a one week-old potato dextrose agar (PDA) culture, and agitating with a glassrod to remove spores. From this suspenSion, 200 Ul was added to PDA plates and incubated for one week at 28±2°C. Conidia were washed off the agar surface by agitating with 10 ml SDW by a glassrod. The suspension was passed through four layers of muslin and washed twice with SDW by centrifugation.

Plants were inoculated at the five to six leaf stage by the root dip method. Plants were depotted and the root system was immersed in spore suspension (10^6 spores ml"^{1}) for five minutes. After inoculation, plants were repotted in the same compost and returned to the glasshouse.

Estimation of Fungal Propagules in Plants

Fusarium propagules (spores and mycelial fragments) in the plants' root tissues were determined at weekly intervals for four weeks after inoculation using the comminution dilution technique based on the method of pegg and Street [16]. Plants were uprooted and the compost removed by a tap water rinse. Roots were further washed by a similar method to that of Banihashemi and De Zeeuw [2]. One gram of root sample was comminuted in 10 ml SDW using a Sorvali Omnimixer at 10000 rpm in an ice bath. Serial dilutions were made for susceptible (10^4), and nonhost (10^2). 0.5 ml suspension was pipetted into sterilized dishes, and 15 ml PCNB-pepton agar (14) which had been held at 50°C was added. The plates were incubated for 2-4 days at 28°C. Ten plates were used for each dilution. *Fol* colonies were expressed as numbers of colony forming units (CFU) per gram of root fresh weight.

In the second experiment, 1 cm of the tap root (2.5 cm above the point of inoculation) and 1 cm of hypocotyl (2 cm below the cotyledonary node) were severed, the cortex removed aseptically, and the pericycle cut into the smallest pieces possible with a razor blade. The degree of colonization in each section was estimated using the same technique as mentioned above, the comminution time for lateral root, tap root, and hypocotyl was 30, 30, and 60 seconds, respectively.

Effect of Inoculum Concentration on Root Colonization

Plants were prepared for inoculation as previously described. Four groups of aubergine plants were inoculated with $5xl0^6$, $5xlO^4$, $5xlO^3$, and $5xlO^2$ spores ml"^{1} of *Fol* race 1 in SDW. The fifth group was treated with SDW. The numbers of CFU g"^{1} root fresh weight, as well as the heights of the plants

[DOR: 20.1001.1.16807073.2000.2.1.1.7]

were determined four weeks following inoculation.

RESULTS

Determination of Fungal Growth in Host and Non-Host Plants.

Results in Table 1, show that the roots of different plants are colonized to various degrees. In the first week after inoculation, similar numbers of propagules were recovered from the roots of host and non-host plants indicating that these plants were colonized to the same extent at the earlier stage. In the third and fourth weeks, after inoculation, larger numbers of propagules were obtained from the roots of susceptible tomato plants than from roots of non-susceptible hosts.

Root colonization of solanaceous plants (aubergine, tomato) and *Chenopodium* increased with time, up to the third week. In the case of the two *Amaranthus* weeds, colonization increased, at least, up to the end of the fourth week when the experiment was terminated. Of all the plant species inoculated, only tomato plants showed symptoms of the disease and wilted. Aubergine was slightly stunted, other weeds remained symptomless.

Table 1. Population of *Fusarium oxysponim f. sp.* lycopersici race 1 in the roots of snsceptible tomato and non-hosts

plant species	No. of propagules[a] g^{-1} fresh rootxl0^3			
	Week after inoculation			
	1	2	3	4
Lycopersicom esculentum	8.8	10.9	68.8	70.5
Solarium melongena	9.5	11.5	15.6a	8a
Chenopodium album	0.2	4.7	13.6a	13.2a
Amaranthus retroflexus	3	2.6a[b]	5a	15.3a
Amaranthus. sp,	1.8	2.4a	2a	7.4a

a Average of three paint replicates.

b Significantly lower number of propagules com pa ted to tomato at the same sampling date LSD at 0.01 level = 7.633

Effect of Inoculum Concentration on Aubergine Colonization

The reaction of aubergine to increasing levels of inoculum concentration is presented in Figure 1. Increasing inoculum concentration had no significant effect on the level of colonization except at $5xl0^6$ (Fig. lb). A similar relationship between the effect of increased inoculum concentration and plant height was observed. Only the highest concentration ($5xl0^6$ spore $ml"^1$), significantly decreased the height of the plants in comparison to the control (Fig. la).

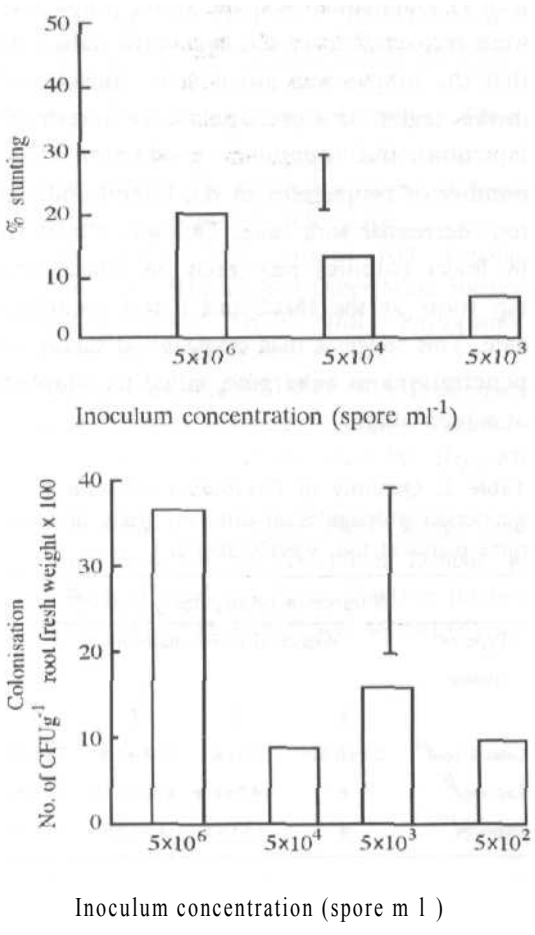

Figure 1. Effect of inoculum concentration of *Fuswvn oxysporum f. sp. lycopersici* on (a) stem height and (b) colonization of aubergine cv. Long Purple four weeks after inoculation. Means of five plant replicates. Vertical bars indicate LSD (P=0.05).

[DOR: 20.1001.1.16807073.2000.2.1.1.7]

Estimation of *Fol* in Aubergine Roots and Shoots

An attempt was made to determine whether different parts of the paint had a similar pattern of colonization.

Results presented in Table 2 show **that** the level of colonization at each date during the four week intervals was always greater on the lateral roots than on the tap root and hypocotyl. The quantity of fungal propagules decreased with increasing distance from the site of inoculation. There was slight vascular browning at the base of the tap root when seen in longitudinal section. Fewer propagules were recovered from the hypocotyl, indicating that the fungus was not able to consolidate in this region as a prerequisite for successful infection and symptom production. The number of propagules in the lateral and lap root decreased with time. This was illustrated by fewer colonies recovered on lateral and tap roots at the third and fourth sampling date. This suggests that many initial successful penetrations in aubergine, failed to establish at a later stage.

Table 2. Quantity of *Fusarium axysporum f. sp,* lycopersici propagules in different parts of aubergine plants at four weekly intervals.

Number of propagulcs g^{-1} tissue[a]				
Type of tissue	Weeks after inoculation			
	1	2	3	4
Lateral rool	2.3±0.34*	17.5±4.1	5.49±1.8	7.1±1.8
Tap rool[b]	0	19,2±1.9	4.80 ± 0.73	5.6±0.9
Hypoctyl[c]	0	5.6±0.8	4.6±0.6	7.2±0.9

a Number of propagules g" fresh lateral rootxlO .

b Number of propagules cm' of tap root cut 2.5 cm above the inoculation point.

c Number of propagules cm^{-1} of hypocotyl cut 1 cm below the cotyledonary node.

D Each point is the average of five plant replicates.

* Standard error

DISCUSSION

This investigation revealed that several nonhost paints, were colonized by *Fusarium* wilt of tomato at various degress and can act as symptomlcss carriers. The difference in colonization between hosts and non-hosts, was because the pathogen failed to colonize the main stele system of the non-host while in the tomato host more propagules were recovered from the roots due to a systemic invasion and proliferation in the vascular system. Other workers [4, 8, 9, 10] also found similar results with wilt fungi such as *Fusarium* and *verdcillium* which infecting many non-host plants.

It has been suggested that the invasion of non-hosts by *Fusarium* which results in root infection, but is not accompanied by the production of any symptoms, is due to the inability of the pathogen to produce toxins, or enzymes, in non-host plants [13,15).

The effect of increasing levels of inoculum concentration on root colonization of aubergine by *Fol,* indicated that only the highest concentration, $5xl0^6$ spore $ml"^1$, significantly reduced the height of the plants by 19% . At the same spore concentration, root colonization was significantly higher when compared to the plants inoculated with lower spore concentrations. A similar result was obtained by Katan [8] who found that concentrations below $lxlO^6$ spores $ml"^1$ were ineffective in decreasing aubergine stem height. At $lxlO^6$ spore $ml"^1$ stem height was reduced by 25% and 18% in two separate experiments.

Woolliama [19] also reported similar stunting to various degrees in weeds, inoculated with *V. dahliae.* With *F. O. f. sp. niveum* both squash and watermelon, showed increased susceptibility as the concentration of inoculum increased from 10^3 to 10^6 microconidia ml'^1 . Cultivars with a high level of resistance were unaffected by increasing inoculum concentration **[11].**

[DOR: 20.1001.1.16807073.2000.2.1.1.7]

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[DOR: 20.1001.1.16807073.2000.2.1.1.7]

J. Agr. Sci. Tech. (2000) Vol. 2: 27-32

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چکیده

در این بررسی تعدادی از گیاهان زراعی و علف‌های هرز جمع‌آوری شده از مزارع گوجه‌فرنگی در استان هرمزگان به عنوان میزبان‌های بدون علائم قارچ *Fusarium oxysporum f.sp. Lycopersici* مورد مطالعه قرار گرفته است. برای این منظور قارچ *F. oxysporum* جدا شده از گیاهان بادمجان *(Solanum melongena)*، تاج خروس *(Amaranthus retroflexus)* و سلمه تره *(Chenopodium album)* تحت شرایط گلخانه به گیاهچه گوجه‌فرنگی و علف‌های هرز از طریق ریشه، مایه‌زنی گردید. قارچ‌های مزبور تنها بر روی گیاهچه گوجه‌فرنگی علائم بیماری را ایجاد نمودند و پس از سه هفته منجر به مرگ آن گردیدند. این نتیجه نشان داد که تمام قارچ‌های جدا شده، *F.o.f.sp. lycopersici* می‌باشد. ریشه سایر علف‌های هرز مانند تاج خروس و سلمه تره و بادمجان با درجات متفاوت مورد حمله قرار گرفتند ولیکن علائمی ایجاد نکردند و در نتیجه نسبت به این قارچ، میزبان‌های بدون علائم محسوب می‌شوند. بنابراین در مناطق آلوده بایستی از کشت متناوب گوجه‌فرنگی و بادمجان خودداری و به روش مطلوبی با علف‌های هرز مبارزه کرد.

[DOR: 20.1001.1.16807073.2000.2.1.1.7]