Induced Resistance to Fusarium wilt of Tomato and Cucumber: Symptoms and Pathogen Proliferation

Henry NELSON^{*,**}, Seiji OUCHI^{*,**}, Tomonori SHIRAISHI^{*}
and Hachiro OKU^{*}

Abstract

Fusarium wilt symptoms were reduced in tomato and cucumber plants when they were inoculated with *Fusarium oxysporum* f.sp. *cucumerinum* (non-pathogenic on tomato) and *F. oxysporum* f.sp. *lycopersici* (non-pathogenic on cucumber), respectively, prior to inoculation with the pathogenic formae speciales. The respective pathogenic forma specialis was isolated at a significantly high frequency from stem sections of symptomless plants which had been pre-inoculated with the respective non-pathogenic forma specialis or a mixture of the pathogenic and non-pathogenic formae speciales, but it was not isolated from symptomless plants which had been inoculated only with the pathogenic forma specialis. The respective non-pathogenic forma specialis was not isolated from stems of tomato or cucumber, irrespective of symptom development. The number of colony forming units per gram fresh weight of stem was less in pre-inoculated plants without symptoms than in pre-inoculated plants with symptoms. The reduction in symptoms observed in these plants following the induction of resistance by pre-inoculation with a non-pathogenic forma specialis may have been due to repressed proliferation of the pathogenic forma specialis.

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Key words: *Fusarium oxysporum* f.sp. *lycopersici*, *Fusarium oxysporum* f.sp. *cucumerinum*, *Lycopersicon esculentum*, *Cucumis sativus*, induced resistance.

INTRODUCTION

Induced resistance is known in viral, bacterial and fungal diseases^{2,4,5,7-9}. It offers an attractive alternative to genetic resistance, particularly for the control of diseases caused by soil-borne pathogens, which often are not amenable to chemical control for environmental and economic reasons. A better understanding of induced resistance is needed to apply it effectively to disease control. One point of controversy is whether the growth or multiplication of the pathogen is inhibited by induced resistance, or whether only the symptoms are suppressed. Discussion of this issue requires knowledge of the behavior of a pathogen in induced plants. Such information concerning vascular wilt pathogens is scant.

We investigated the roles of two formae speciales of *Fusarium oxysporum* as a pathogen and as a non-pathogenic inducer of resistance in two plant species, tomato and cucumber. We report herein on the isolation frequency and proliferation of these formae speciales in their respective hosts.

MATERIALS AND METHODS

Tomato (*Lycopersicon esculentum* Mill. 'Fukuju 2') and cucumber (*Cucumis sativus* L. 'Shogoin-Fushinari') plants were grown, in a glasshouse at ambient temperature, from seed in vermiculite.

* College of Agriculture, Okayama University, 1-1-1 Tsushimanaka, Okayama 700, Japan 岡山大学農学部

** Present address: Faculty of Agriculture, Kinki University, 3327-204 Nakamachi, Nara 631, Japan 現在: 近畿大学農学部

Tomatoes were first inoculated at the four-leaf stage, and cucumbers, at the three-leaf stage. Water-suspensions of bud-cells⁸⁾ (1×10^6 cells/ml) from 9-day shake cultures of *F. oxysporum* f.sp. *lycopersici* (Sacc.) Snyder et Hansen and *F. oxysporum* f.sp. *cucumerinum* Owen were used as inoculum. Plants were pre-inoculated by pouring 20 ml of a bud-cell suspension of the respective non-pathogenic forma specialis or a 1:1 mixed suspension (5×10^5 cells/ml each, of the non-pathogenic and pathogenic formae speciales) around the base of each plant. Control plants received water. The plants were challenge-inoculated with the respective pathogenic forma specialis an average of 4 days (range: 2-7 days) later.

The plants were examined for internal symptoms which were rated on the basis of vascular browning as: 0=no browning, 1=browning in the roots only, 2=browning in the lower stem, and 3=browning in the upper stem⁶⁾. The node of the first true leaf was examined for the presence of both formae speciales at various intervals after the first inoculation, tomato: 13-51 days; cucumber: 20-54 days. A surface-sterilized stem section surrounding the node was aseptically sliced, and a homogenate of 1 g of the slices in 5 ml of sterile distilled water was plated on Komada's selective medium (described in Ref. 7). Homogenates of plants with symptoms rated at 2 or 3 were diluted tenfold. After culturing for 10 days, colonies were counted. Although it was possible to distinguish the two isolates by the color and morphology of colonies on Komada's medium, individual colonies were transferred to potato dextrose agar slants and identified by observing the macroconidia 6 weeks later.

The data of 11 experiments with four plants for each treatment are presented, except for the data of isolation attempts when no vascular discoloration was observed and the pathogenic forma specialis was not found in any plant of any treatment (seven cucumber plants of each treatment). The data of uninoculated controls (no disease observed) are not shown. The significance of pre-inoculation treatment effects on internal symptoms was analyzed by least significant difference test after analysis of variance. Differences in the distribution of isolation frequencies among the treatments between plants with and without symptoms were tested for significance by χ^2 test ($p < 0.05$). In order to minimize the influence of possible changes in the extent of colonization over time, only the data of a 16-day period (tomato, 23-38 days after inoculation; cucumber, 30-45 days) was used to calculate the average number of colony forming units (cfu). No trend for cfu's to increase or decrease in number during this time period was noted. The significance of differences in the numbers of cfu's between pre-inoculated plants with symptoms and those without symptoms was tested by the Mann-Whitney *U* test ($p < 0.05$).

RESULTS

As Homma and Ohata⁹⁾ have reported, pre-inoculation with *F. oxysporum* f.sp. *cucumerinum* was effective in reducing vascular browning symptoms caused by *F. oxysporum* f.sp. *lycopersici* in tomato plants (Table 1). Protection was achieved even if the first inoculum was a mixture of the non-pathogenic and pathogenic formae speciales. Pre-inoculation with *F. oxysporum* f.sp. *lycopersici* was effective in reducing vascular browning symptoms caused by *F. oxysporum* f.sp. *cucumerinum* in cucumber plants,

Table 1. Percentage of plants with internal symptoms of each degree

Host	Inoculation protocol ^{a)}	Internal symptoms ^{b)}				F	(n)
		0	1	2	3		
Tomato	cuc → lyc	59	2	5	34	15.5 ^{c)}	(44)
'Fukuju 2'	mix → lyc	43	5	5	48	5.96 ^{d)}	(44)
	H ₂ O → lyc	18	2	14	66	—	(44)
Cucumber	lyc → cuc	65	11	16	8	8.24 ^{c)}	(37)
'Shogoin-	mix → cuc	51	5	11	32	0.86	(37)
Fushinari'	H ₂ O → cuc	38	8	19	35	—	(37)

a) The first treatment is indicated to the left of the arrow, and the challenge, to the right. Abbreviations: cuc, *F. oxysporum* f.sp. *cucumerinum*; lyc, *F. oxysporum* f.sp. *lycopersici*; mix, 1:1 mixture of the two formae speciales.

b) See MATERIALS AND METHODS for details on the ranking of internal symptoms.

c), d) Significantly different from plants pre-treated with water at $p < 0.01$ and at $p < 0.05$, respectively.

Table 2. Percentage of plants with symptoms and without symptoms from which the respective pathogenic forma specialis was isolated^{a)}

Host	Inoculation protocol ^{b)}	Internal symptoms		Total
		0	1-3 ^{c)}	
Tomato	cuc → lyc	18	37	55 (21/38) ^{d)}
'Fukuju 2'	mix → lyc	14	54	68 (25/37)
	H ₂ O → lyc	0	78	78 (28/36)
Cucumber	lyc → cuc	15	38	54 (14/26)
'Shogoin-Fushinari'	mix → cuc	21	45	66 (19/29)
	H ₂ O → cuc	0	72	72 (18/25)

a) The distribution of frequencies of isolation of the pathogenic formae speciales between plants with symptoms and plants without symptoms differed significantly according to treatment ($p < 0.05$).

b) Abbreviations are as in Table 1.

c) Plants with internal symptoms rated at 1 to 3.

d) In parentheses are the number of positive isolations/the total number of isolation attempts.

Table 3. Number of colony forming units per gram fresh weight of stem tissue at the first node of symptomless plants and plants with symptoms

Host	Inoculation protocol ^{a)}	Internal symptoms	
		0	1-3
Tomato	cuc → lyc	309 ± 165 ^{b)}	989 ± 466
'Fukuju 2'	mix → lyc	349 ± 171	794 ± 569
	H ₂ O → lyc	—	583 ± 170
Cucumber	lyc → cuc	38 ± 34	847 ± 661
'Shogoin-Fushinari'	mix → cuc	43 ± 25	1248 ± 956 ^{c)}
	H ₂ O → cuc	—	789 ± 368

a) Abbreviations are as in Table 1.

b) The data are given as the mean ± standard error.

c) Significantly different from symptomless plants of the same inoculation protocol at $p < 0.05$.

but not if the non-pathogenic and pathogenic formae speciales were mixed.

F. oxysporum f.sp. *lycopersici* was not isolated from a symptomless tomato plant that had not been pre-inoculated with *F. oxysporum* f.sp. *cucumerinum* (Table 2). Likewise, *F. oxysporum* f.sp. *cucumerinum* was not isolated from a symptomless cucumber plant that had been inoculated only with the pathogenic formae speciales. In contrast, 20% (5 of 25 isolations) to 33% (7 of 21 isolations) of the total isolations of the respective pathogenic formae speciales were from symptomless plants when plants had been pre-inoculated with the non-pathogenic forma specialis or the mixture. The overall isolation frequency disregarding symptoms increased in both hosts in the order of pretreatment with the non-pathogenic forma specialis < mixture of formae speciales < water. Neither non-pathogenic forma specialis was found in the stem at the first node of its non-host, irrespective of vascular discoloration and irrespective of the presence of the pathogenic forma specialis.

Less cfu's per gram fresh weight of nodal tissue were isolated from symptomless plants than from plants expressing symptoms within the pre-inoculation treatment groups (Table 3). Despite the great variation in numbers of isolated cfu's, significance was recognized in cucumber plants which had been pre-inoculated with the mixture of pathogenic and non-pathogenic formae speciales between plants without symptoms (43 ± 25) and those with symptoms (1248 ± 956). No statistically significant difference in cfu's per gram of tissue was recognized between pre-inoculated plants with symptoms and water pre-treated plants with symptoms.

DISCUSSION

A suppression of symptoms due to systemic acquired resistance which was not accompanied with a reduction in multiplication of the pathogen has been reported in bacterial⁴⁾ and viral diseases.⁹⁾ This phenomenon may not be the case in all host-parasite interactions. Ishiba *et al.*⁷⁾ reported that preinoculation with a hypovirulent strain of *F. oxysporum* f.sp. *cucumerinum* reduced the number of anthracnose lesions on the leaves of cucumber plants. Similar results were obtained by Biles and Martyn²⁾ on watermelon. However, Gessler and Kuć⁵⁾ found that pre-inoculation of cucumber with a wilt pathogen or non-pathogen did not induce resistance against *Colletotrichum lagenarium*. Gessler and Kuć evaluated induced resistance a week after all diseased control plants had died to assure that any protective effect observed would not be due to a reduced rate of disease development. Their results suggest that symptoms may be reduced or delayed by induced resistance while establishment of the pathogen is not stopped.

In the present study pre-inoculation of tomato and cucumber plants with the respective non-pathogenic forma specialis, while reducing symptoms, did not prevent the invasion of the host by the pathogenic forma specialis. However, penetration and colonization of the vessels are distinct phases in the development of wilt diseases¹⁾, and as Brandt *et al.*³⁾ have shown in the mint-*Verticillium* system, disease resistance is based on the limitation of proliferation of the pathogen in the stem. In our study fewer propagules were isolated from pre-inoculated plants in which resistance was successfully induced (no symptom expression) than from pre-inoculated plants in which resistance was not induced (symptoms expressed). This result suggests that the induced resistance observed in the present study was due to the repression of fungal proliferation.

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和 文 摘 要

Henry NELSON・大内成志・白石友紀・奥 八郎：トマトおよびキュウリのフザリウム病における誘導抵抗性一病徴ならびに病原菌の増殖

あらかじめ非病原性分化型のフザリウム病菌を単独または病原性分化型菌と混合接種したトマトとキュウリでは、多くの場合維管束褐変が起らなかった。しかし、これら無病徴植物の茎の磨砕液からは病原菌が高頻度に分離された。ただし、いずれの場合にも、誘導菌として用いた非病原性分化型菌は分離されなかった。第一節間茎部組織の病原型菌数(cfu/g f.w.)について比較したところ、抵抗性が誘導され褐変の認められなかった植物の病原型菌数は褐変を伴った植物のそれより少なかった。この結果から、これらの系における誘導抵抗性は病原菌侵入後の増殖抑制によるものと考えた。