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Invasiveness of *Pseudomonas solanacearum* in tomato, eggplant and pepper: a comparative study

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Abstract. The colonization of eggplant, pepper and tomato by *Pseudomonas solanacearum* was compared. Latent infections were observed in pepper and eggplant, indicating that this phenomenon was general in the main hosts of *P. solanacearum.* In eggplant and pepper, as in tomato, resistance did not arise from a resistance to root colonization by the bacteria. In tomato and eggplant, resistance mechanisms appeared to be similar: spread of *P. solanacearum* was limited in resistant cultivars. In contrast, in pepper this limitation was not observed and plants appeared more tolerant to high bacterial populations than tomato and eggplant.

Introduction

Bacterial wilt caused by *Pseudomonas solanacearum* is a devastating disease particularly on Solanaceous crops in tropical and subtropical areas [Matos et al., 1990; Winstead and Kelman, 1960; Narayanan and Gopinathan Nair, 1983; Digat and Escudié, 1967]. To control this soilborne vascular pathogen, cultural practices and chemicals are insufficient [Daly, 1986; Enfinger et al., 1979]. The most widespread measure is therefore the use of resistant cultivars [Hayward, 1991].

Knowledge of disease resistance mechanisms is of great concern to improve breeding programs for resistance. Latent infections have been demonstrated in bacterial wilt resistant tomato cultivars [Prior et al., 1990; Grimault and Prior, 1993], in potato [Ciampi et al., 1980] and in some weeds [Hayward, 1987; Caudron et al., 1992]. The study of resistance in tomato and potato to wilt showed that the spread of *P. solanacearum* in the stems of resistant cultivars was limited compared to susceptible ones [Grimault et al., 1993; Bowman and Sequeira, 1982]. So, among the four most widely grown Solanaceous species (tomato, potato, eggplant and pepper), the characteristics of plant colonization by *P. solanacearum* were comparable in tomato and potato. By contrast we did not find information on eggplant and pepper resistance mechanisms. To verify if the characteristics of plant colonization by *P. solanacearum* can be generalized to the different hosts of the bacteria, our study focused on two of the main

Solanaceous hosts of *P. solanacearum*: eggplant and pepper. We compared the colonization of susceptible and resistant cultivars of these two hosts with tomato colonization.

Material and methods

Plants

Three tomato cultivars were selected: Floradel (susceptible to bacterial wilt), Hawaï 7996 and Caraïbo (resistant), as well as pepper cultivars Yolo Wonder and Piperade (susceptible) and Narval (resistant) [Kaan and Anaïs, 1977], and eggplant cultivars SM6 and Kalenda (resistant), Ceylan (moderately resistant) and Aranguez (susceptible) [Daly, 1986; Herbert, 1985].

Seeds were sown in vapor disinfected soil. Plants at the cotyledonary stage were transplanted in 7 cm × 7 cm pots. Plants of 3 weeks-old for tomato and 5 weeks-old for eggplant and pepper were transplanted in 4 litre pots. In a preliminary experiment 50 plants of the susceptible cultivars Floradel, Yolo Wonder and Aranguez were used. In a second experiment, two replications of 15 plants were used. The experiments were carried out in an insect-proof glasshouse.

Bacterial strains and inoculation

The bacteria were cultivated on Kelman's medium [Kelman, 1954]. Inoculum was prepared from bacteria grown on tetrazolium chloride-free medium. Cells were harvested, after 48 h culture at 30 °C, by flooding plates with sterile distilled water and inoculum was diluted to 2×10^7 cfu/ml by measuring the optical density (OD 650 nm = 0.01 corresponding to 10^7 cfu/ml).

Different strains of *P. solanacearum* with a broad host range were tested on eggplant, pepper and tomato, to select the more aggressive strain. These strains, classified as race 1 (8217, 8218, 8222, 8227 and 8225), were natural mutants resistant to streptomycin (200 μg/ml) and/or to rifampicin (50 μg/ml) of strains isolated in Guadeloupe (respectively GT1, GT4, GA2 and GA4 for 8227 and 8225). Their characteristics have been previously reported [Prior and Steva, 1990]. Ten plants per susceptible cultivar and per strain were infected at 4 weeks-old for tomato and 6 weeks-old for eggplant and pepper. This stage corresponds to the age of plants when they are transplanted into the field in natural growing conditions. Plants were infected, by inserting a scalpel into the soil at 1 cm from the stem, and pouring 2 ml of inoculum in the soil. The percentage of wilt was determined fifteen days after inoculation.

In the second experiment, fifteen plants per cultivar (resistant or susceptible) were simultaneously inoculated by root injury as previously described. Two repetitions were carried out.

Sampling and analysis
In the second experiment, wilted plants were sampled during the course of the experiment. Stems from wilted plants were washed under tapwater and surface disinfected with alcohol. Fragments of 2 cm from the hypocotyl and midstem were sampled and weighed. Symptomless plants were sampled 21 days after inoculation when bacterial wilt reached its maximum. Secondary roots were removed and taproots were washed under tapwater. Fragments of 2 cm from the taproot, hypocotyl and midstem were sampled as previously described. Taproots were crushed in 5 ml sterile distilled water and extracts were filtered through gauze. Fragments of hypocotyl and midstem were placed in 4 ml of sterile distilled water, overnight at 10 °C to allow the sedimentation of the bacteria while growth was arrested.

Extracts were plated using the Spiral system [Jalenques, 1988] on selective medium (Kelman's medium +50 μg/ml rifampicin +50 μg/ml bacitracin). The medium was supplemented with cycloheximide (50 μg/ml) to avoid fungal growth. Bacteria were counted after 48 h of culture and densities were expressed as colony forming unit (cfu) per gram of fresh matter (cfu/g FM) and log transformed (log cfu/g FM). Average densities were calculated only on plants colonized over 10^2 cfu/ml (Spiral detection lower limit) and compared by a variance analysis (Fisher's test) [Snedecor and Cochran, 1971].

Results

Bacterial wilt
Table 1 shows that the strain 8227 was the more aggressive on the three hosts. This strain was also resistant to bacitracin (50 μg/ml) and it was selected for the experiment.

Table 2 summarizes the percentages of wilting observed on the different hosts 21 days after inoculation by the strain 8227. The resistant cultivars

Table 1. Aggressiveness of five strains of *P. solanacearum* with a broad host range on susceptible cultivars of tomato, pepper and eggplant

	Disease rating		
Strain	Tomato	Eggplant	Pepper
8217	7[a]	1	0
8218	7	1	0
8227	7	5	7
8222	0	0	0
8225	1	0	0

[a] number of wilted plants out of 10, 15 days after inoculation

Hawaï 7996, Caraïbo, Narval and SM6 did not wilt. In the susceptible cultivars, the percentages of wilting were relatively low (from 50% to 56.6%) except for Aranguez (70%). The resistant eggplant cultivars wilted at low frequencies (13.3% for Kalenda and 30% for Ceylan). The bacterial densities counted in wilted plants are presented in Table 2. All wilted plants were colonized at the hypocotyl and midstem, and bacterial densities were high (approximatively 10^9 cfu/g FM). No significant difference was observed among the cultivars at midstem. Only Floradel was significantly less colonized ($P = 0.02$) at the hypocotyl level. All wilted plants were sampled except for Floradel because a rapid maceration of tissues of young plants at the beginning of the experiment did not allow the sampling of all wilted plants.

Table 2. Bacterial densities counted in wilted plants of tomato, pepper and eggplant, inoculated with the strain 8227

Cultivar	% wilting[1]	Bacterial density[2]	
		hypocotyl	midstem
Floradel	56.7	9.28 ± 0.32^a	9.37 ± 0.33^a
Hawaï 7996	0	nd	nd
Caraïbo	0	nd	nd
Yolo Wonder	50	9.69 ± 0.16^b	9.16 ± 0.20^a
Piperade	50	9.82 ± 0.10^b	9.50 ± 0.13^a
Narval	0	nd	nd
Aranguez	70	9.92 ± 0.31^b	9.19 ± 0.30^a
Ceylan	30	9.77 ± 0.25^b	9.05 ± 0.19^a
Kalenda	13.3	10.0 ± 0.07^b	9.80 ± 0.11^a
SM6	0	nd	nd

[1] on 30 plants.
[2] expressed as log cfu/g fresh matter. nd = not determined. Values with the same superscript are not significantly different according to the Fisher's test ($P = 0.02$ at the hypocotyl and $P = 0.12$ at midstem).

Bacterial spread and multiplication in symptomless plants

All cultivars were invaded at taproot (Table 3). The bacterial densities could not be determined at taproot level because of important contaminations on the Petri dishes. In pepper, the frequency of taproot colonization was 100% for all non wilted cultivars. By contrast in eggplant and tomato, differences were observed between resistant and susceptible cultivars. Susceptible cultivars had higher colonization frequencies than the resistant ones. From taproot to midstem, a decrease in colonization frequencies was observed in tomato and eggplant, except for the tomato cultivar Floradel. In pepper, this decrease was not observed.

Bacterial densities observed in the hypocotyl were very high in pepper (10^7 to 10^8 cfg/g FM) compared to eggplant and tomato (10^3 to 10^6 cfu/g

Table 3. Colonization of non wilted plants of tomato, pepper and eggplant by *Pseudomonas solanacearum*

Hosts	Cultivar	Taproot	Hypocotyl		Midstem	
		Col. Freq.[1]	Density[2]	Col. Freq.[1]	Density[2]	Col. Freq.[1]
Tomato	Floradel	11/13 (84.6%)	6.15 ± 0.85^a	10/13 (77%)	4.92 ± 0.88^a	10/13 (77%)
	Caraïbo	14/30 (46.6%)	4.15 ± 0.70^a	9/30 (30%)	3.18 ± 0.36^a	6/30 (20%)
	Hawaï 7996	7/29 (24%)	5.44^a	1/29 (3.5%)	nd	0/29 (0%)
Pepper	Y. Wonder	15/15 (100%)	8.37 ± 0.36^a	15/15 (100%)	8.80 ± 0.37^b	15/15 (100%)
	Piperade	15/15 (100%)	7.37 ± 0.78^a	13/15 (86.6%)	7.20 ± 0.69^a	13/15 (86.6%)
	Narval	30/30 (100%)	8.24 ± 0.30^a	30/30 (100%)	6.70 ± 0.43^a	30/30 (100%)
Eggplant	Aranguez	9/9 (100%)	3.64 ± 0.46^a	7/9 (78%)	2.65	1/9 (11%)
	Ceylan	15/21 (71.4%)	5.38 ± 0.96^a	7/21 (33%)	7.53 ± 0.28	2/21 (9.5%)
	Kalenda	8/25 (32%)	3.36 ± 0.98^a	2/25 (8%)	5.7	1/25 (4%)
	SM6	7/30 (23.3%)	2.92 ± 0.95^a	3/30 (10%)	4.64	1/30 (3.3%)

[1] number of colonized plants/number of non wilted plants sampled. Colonization percentages were put in brackets.

[2] expressed as log cfu/g fresh matter. Values with the same superscripts are not significantly different according to the Fisher's test. Analysis were carried out separately for each host at the different sampling levels. Tomato: $P = 0.089$ (hypocotyl), $P = 0.16$ (midstem). Pepper: $P = 0.32$ (hypocotyl), $P = 0.02$ (midstem). Eggplant: $P = 0.12$ (hypocotyl). Averages values calculated on two repetitions of 15 plants.

FM). Statistical analysis was carried out separately for each host at the different sampling levels. In the different hosts of *P. solanacearum*, no significant differences were shown between bacterial densities encountered at the hypocotyl in susceptible and resistant cultivars. At midstem, a significant difference was observed between the pepper cultivars: the resistant cultivar Narval was less colonized than the susceptible one Yolo Wonder. No significant difference was observed between the bacterial densities encountered at midstem in the tomato cultivars. In eggplant, the statistical analysis was not carried out because of the low number of colonized plants.

In susceptible pepper cultivars, bacterial densities were relatively stable between the hypocotyl and midstem whereas in the resistant cultivar, a decrease of bacterial density was observed. In tomato, a similar decrease was observed in all cultivars, and in eggplant, the low number of colonized plants did not allow a good estimation of bacterial densities.

Discussion

The strain 8227 was chosen for its broad host range despite its low aggressiveness as shown by the percentages of wilt obtained. In Floradel, macerated plants were not sampled and bacterial densities were underestimated as compared to the other cultivars because only the plants beginning to wilt were sampled. The comparable bacterial populations at the hypocotyl and midstem of wilted plants indicated that the threshold above which wilt occurs was similar in the different cultivars of the three hosts of *P. solanacearum*. This is consistent with results obtained on a range of tomato cultivars differing in resistance to bacterial wilt [Grimault et al., 1993].

The different pepper and eggplant cultivars presented latent infections (symptomless colonized plants) as for tomato [Prior et al., 1990; Grimault and Prior, 1993]. We wish to point out that plants were inoculated by root injury, which could promote the penetration of bacteria. The inoculation technique we used reflected the colonization of tomato by *P. solanacearum* since recent studies have demonstrated that wilting frequencies were similar in plants inoculated with and without root injury (J.G. Elphinstone pers. comm.). Moreover, Khan et al. [1988], also observed the presence of bacteria in resistant eggplant cultivars inoculated without root injury. So, bacteria are able to penetrate in eggplant roots by natural openings as in tomato [Schmit, 1978; Kelman and Sequeira, 1965]. The penetration of *P. solanacearum* was not studied in pepper. Nevertheless, in natural conditions, the injuries caused to roots by the transplantation of plants in the field promote the penetration of the bacteria. So, the presence of latent infection appears to be a general phenomenon not only in tomato cultivars [Grimault et al., 1993] but also in pepper and eggplant. Moreover, in eggplant and pepper, resistance did not arise from a resistance to root colonization by *P. solanacearum* since taproots were colonized at high frequencies.

Tomato cultivars resistant to bacterial wilt showed a reduction of colonization from taproot to midstem. This confirmed the results obtained with another strain of *P. solanacearum* [Grimault et al., 1993] and indicated that the limitation of *P. solanacearum* spread was also observed in eggplant: the colonization at the different levels was lower (density and frequency) in resistant cultivars. So, we assume that the result of resistance mechanisms of tomato and eggplant might be similar. By contrast, in pepper, the result of resistance mechanisms seem to be different: no reduction of colonization was observed in the resistant cultivar. At midstem, the bacterial densities encountered in Narval, although significantly lower than in the susceptible cultivar Yolo Wonder, were very high. Pepper seemed therefore to be more tolerant than tomato and eggplant to high bacterial populations.

The presence of latent infections in the principal hosts of *P. solanacearum* may have important epidemiological consequences since growing tolerant cultivars of these three hosts of *P. solanacearum* in the field would increase the inoculum level in soils. This fact should be kept in mind for crop rotations [Melton, 1991; Sohi et al., 1981]. It would be also important to manage weeds as numerous species were proven to be symptomless carriers [Caudron et al., 1992; Hayward, 1987].

Colonization criteria were proposed as additional criteria for breeding programs on tomato [Grimault et al., 1993]. This study demonstrated that it would be also useful to take into account these criteria for breeding resistant eggplant cultivars. By contrast in pepper, colonization criteria did not permit differentiation of resistant cultivars from susceptible ones, and resistance mechanisms are presently not defined.

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