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RESISTANCE TO *CORYNEBACTERIUM MICHIGANENSE* IN TOMATO GENOTYPES

N. A. M. VAN STEEKELENBURG

Research Institute for Plant Protection (IPO) Wageningen, seconded to the Glasshouse Crops Research and Experiment Station, P.O. Box 8, 2670 AA Naaldwijk, the Netherlands

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SUMMARY

The highest level of resistance to *Corynebacterium michiganense* was found in 'Irat L3' and 'Okitsu Sozai 1-20'. Resistance was partial and symptomless plants proved to be carriers of the disease. The resistance is at least partially based on a diminished multiplication of the pathogen in the host plant.

INTRODUCTION

Bacterial canker caused by *Corynebacterium michiganense* pv. *michiganense* (SMITH) JENSEN occurs in many tomato growing areas of the world (STRIDER, 1969). Symptoms of the disease are described by many authors, among others STRIDER (1969). Birds-eye spots on fruits and white spots or blisters on leaves and stems result from bacterial colonisation of the surface tissues of the tomato plant and fruit. Necrotic lesions in the leaves, marbling of fruits and wilting, often unilaterally, are systemic symptoms resulting from bacterial invasion of the vascular tissue. The disease can hardly be controlled other than by hygienic measures. A study was undertaken to investigate the perspectives of breeding for resistance. Preliminary tests were carried out to develop a fast and reliable inoculation technique.

Many techniques, reviewed by STRIDER (1969) and LATERROT et al. (1978) have been devised for inoculating tomato plants with *C. michiganense*. As systemic infection causes the most serious economic losses under commercial conditions, methods were compared in which the pathogen is introduced directly into the vascular system after wounding. Several cultivars and accessions of tomato obtained via the Institute for Horticultural Plant Breeding (IVT) at Wageningen were tested as young and as mature plant. The multiplication of the pathogen in susceptible and partially resistent cultivars was studied to investigate the nature of resistance.

MATERIALS AND METHODS

Cultures of *C. michiganense* were grown on yeast extract (0.5%) peptone (1%) glucose (0.5%) agar (2%) for 3 to 5 days at 25°C. Inoculum was prepared by suspending the bacteria in a saline solution (0.85% NaCl). With the aid of a haemacytometer the

Table 1. List of *Lycopersicon* cultivars/accessions tested for resistance to *Corynebacterium michiganense*.

Cultivar	Species	Origin	Author
Moneymaker*	*L. esculentum*	Netherlands	
Sonatine*	*L. esculentum*	Netherlands	
Florida MH-1**	*L. esculentum*	USA	BOELEMA, 1980
Utah 20 (Pl 344102)**	*L. pimpinellifolium*	USA	THYR, 1968; DE JONG & HONMA, 1976
Bulgaria 12 (Pl 330727)**	*L. esculentum*	Bulgaria	ELENKOV, 1965
Irat L3**	*L. esculentum*	Martinique	LATERROT, 1980
Okitsu Sozai 1-20**	*L. esculentum*	Japan	KURIYAMA & KUNIYASU, 1974 LATERROT, 1980.

* Susceptible control.
** Resistant according to the authors mentioned.

number of cells per ml was adjusted to 10^7 unless stated otherwise.

Seedlings of tomato were transplanted into 12 cm plastic pots two weeks after sowing. The cultivars tested are listed in Table 1. Inoculation was carried out between the fourth and fifth leaf stage, when plants were 10 to 15 cm high. Every week the number of diseased plants (= plants with wilting symptoms) and, as indication of the degree of attack, the number of wilted leaflets of the five oldest leaves were counted.

Experiments were conducted in a glasshouse throughout several years with the temperature thermostatically set at 18/22 °C (night/day). The temperature may have risen higher with sunshine.

INOCULATION TECHNIQUES

The instruments used for inoculation were contaminated by dipping them into the bacterial suspension before every inoculation. The following techniques were used:
- pricking the stem near the first leaf with a scalpel blade.
- intersecting one vascular bundle of the stem near the first leaf with a scalpel blade. With this method it was necessary to bandage the stem at the inoculation site with 'sellotape' to prevent collapsing of plants.
- cutting the petiole of the first true leaf near the stem with a pair of scissors.
- clipping the top of the plant with a pair of scissors.
- dipping the root system into the inoculum after cutting the roots to half length.

The first symptoms of wilting were observed seven days after inoculation. Symptom expression was slowest with root inoculation, followed by cutting the leaf petiole (Tables 2 and 3). As this last method was easy and rapid to perform it was used in the resistance tests.

YOUNG PLANT RESISTANCE

Two experiments with young plants were carried out. In the first experiment disease development on 'Irat L3' and 'Okitsu Sozai 1-20' was delayed and about half the number of plants of these cultivars remained symptomless (Table 4). The differentiation between resistant and susceptible cultivars was more pronounced with the percent-

Table 2. Percentages diseased plants of tomato cv. Moneymaker one, two, three and four weeks after inoculation by different methods with *Corynebacterium michiganense* (16 plants per treatment).

Inoculation method	Percentage diseased plants after			
	1w	2w	3w	4w
intersecting vascular bundle	56	100	100	100
clipping top	40	100	100	100
pricking stem	40	100	100	100
cutting leaf petiole	7	94	100	100
dipping root system	0	19	38	75

Table 3. Percentage wilted leaflets of tomato cv. Moneymaker one, two, three and four weeks after inoculation by different methods with *Corynebacterium michiganense* (16 plants per treatment with 5 leaflets per plant).

Inoculation method	Percentage diseased plants after			
	1w	2w	3w	4w
intersecting vascular bundle	7	46	77	96
clipping top	4	28	74	100
pricking stem	3	44	89	98
cutting leaf petiole	1	14	39	76
dipping root system	0	4	8	36

age of diseased leaflets than with the percentage of diseased plants.

In the second experiment (Table 5), disease development was faster than in the first experiment. When plants were inoculated with a concentration which was a hundred times lower, disease development was somewhat slower on most cultivars. The number of diseased plants of 'Irat L3' and 'Okitsu Sozai 1-20' did not increase after four weeks. The symptomless plants remained symptomless even after three months.

Table 4. Percentages of diseased plants and percentages of wilted leaflets of the five oldest leaves of tomato cultivars inoculated in the fifth leaf stage with *Corynebacterium michiganense* (15 plants per treatment).

Cultivar	% diseased plants after		% wilted leaflets after	
	2w	4w	2w	4w
Moneymaker	20	75	2	47
Sonatine	20	93	2	59
Florida MH-1	13	93	1	57
Bulgaria 12	20	93	1	56
Utah 20	7	87	1	55
Irat L3	0	53	0	10
Okitsu Sozai 1-20	0	47	0	12

Table 5. Percentages of diseased plants of tomato cultivars inoculated in the fifth leaf stage with two concentrations of *Corynebacterium michiganense* (15 plants per treatment).

Cultivar	% diseased plants after (10^7 bacteria per ml)		% diseased plants after (10^5 bacteria per ml)	
	2w	4w	2w	4w
Moneymaker	80	100	67	100
Sonatine	93	100	67	100
Florida MH-1	33	100	47	100
Utah 20	73	100	67	100
Bulgaria 12	47	100	–	–
Okitsu Sozai 1-20	20	73	13	40
Irat L3	0	13	–	–

MATURE PLANT RESISTANCE

Fruit bearing five-month old plants were inoculated by dripping 0.02 ml of the bacterial suspension in the wound made with a knive at the tenth leaf axil of the stem.

The first wilting symptoms were observed at the same time on 'Moneymaker', 'Sonatine' and 'Bulgaria 12', about four weeks after inoculation. 'Okitsu Sozai 1-20' and 'Irat L3' were the most resistant cultivars both with respect to incubation period and number of symptomless plants after 12 weeks (Table 6).

PRESENCE OF C. MICHIGANENSE IN STEM TISSUE

At different times after inoculating plants in the fifth leaf stage the stems of some plants were cut in nine segments of equal length, beginning at the inoculation site to the top.The lower, middle and upper part of the stem were weighed, surface disinfected by burning them with alcohol and cut into pieces a few millimeters long. The stem pieces of each part were put in 10 ml of saline solution for at least 1 h. For qualitative assays this solution was plated directly on yeast peptone glucose agar. For quantitative assays a dilution series was made and appropriate dilutions were plated. After incubation for three to five days at 25 °C colonies were counted.

Table 6. Number of diseased mature plants of tomato cultivars four, six, eight, ten and twelve weeks after inoculation with *Corynebacterium michiganense*.

Cultivar	Number of plants tested	Number of diseased plants after				
		4w	6w	8w	10w	12w
Moneymaker	8	2	7	7	8	8
Sonatine	10	2	5	9	9	9
Bulgaria 12	5	2	2	2	2	3
Utah 20	9	0	3	6	8	9
Okitsu Sozai 1-20	9	0	1	1	2	5
Irat L3	7	0	0	0	3	3

Table 7. *Corynebacterium michiganense* detected (+) or not detected (−) in stem tissue of tomato cultivars four weeks after inoculation.

Cultivar	Degree of wilting	Part of the stem		
		lower	middle	upper
Sonatine	9/9*	+	+	+
	7/9	+	+	+
	4/9	+	+	−
Florida MH-1	7/9	+	+	+
	5/9	+	+	+
	3/9	+	+	+
Utah 20	7/11	+	+	−
	5/11	+	+	−
	3/11	+	−	−
Bulgaria 12	7/9	+	+	+
	5/9	+	+	+
	4/9	+	+	−
Irat L3	1/9	+	+	+
	0/9	+	+	+
	0/9	+	+	−
Okitsu Sozai 1-20	9/9	+	+	+
	2/9	+	+	−
	0/9	+	−	−

*Number of diseased leaves/number of fully developed leaves.

Table 8. Logarithm of numbers of colony-forming units of *Corynebacterium michiganense* per gram fresh stem tissue of susceptible and resistant tomato cultivars.

Cultivar	Stem part	Weeks after inoculation		
		6	8	12
Moneymaker	lower	15.2	12.5	
(wilted)	middle	15.3	11.5	
	upper	12.0	10.9	
Irat L3	lower	9.2	7.3	7.3
(symptomless)	middle	9.3	5.5	6.7
	upper	< 7.0	1.1	1.2
Okitsu-Sozai 1-20	lower	7.9	6.0	6.3
(symptomless)	middle	7.9	2.3	5.9
	upper	< 7.0	2.6	2.0

In many plants *C. michiganense* could be found in higher parts of the stem than to where wilting had progressed (Table 7). Bacteria could be isolated from symptomless plants, sometimes even from the top (Tables 7 and 8). The number of bacterial cells per gram fresh stem tissue did not increase between 6 and 12 weeks after inoculation (Table 8). In symptomless plants of resistant cultivars the number of bacteria was considerably reduced in comparison with a wilted plant of a susceptible cultivar. The concentration of bacteria was lowest in the upper plant parts.

DISCUSSION AND CONCLUSIONS

Cutting the petiole of the first true leaf of young plants with a contaminated pair of scissors is a fast and easy inoculation technique in screening for resistance to systemic infection of *C. michiganense*. On young and on mature plants the same difference in susceptibility between the cultivars tested was found, which suggests that screening on young plants is reliable. The resistance in 'Florida MH-1' (BOELEMA, 1980), 'Utah 20' (DE JONG & HONMA, 1976; THYR, 1968) and 'Bulgaria 12' (ELENKOV, 1965; THYR, 1971; DE JONG & HONMA, 1976; BOELEMA, 1980) could not be confirmed or was of too little value.

'Irat L3' and 'Okitsu Sozai 1-20' showed the highest level of resistance and confirmed results obtained by LATERROT (1980). However, the resistance was partial and symptomless plants proved to be carriers of the pathogen. In symptomless plants of 'Irat L3' and 'Okitsu Sozai 1-20' the number of bacteria was about 10^6 times lower than in the susceptible 'Moneymaker'. Neither the number of diseased plants of resistant cultivars, nor the number of bacteria per plant increased after 6 weeks after inoculation of young plants. THYR (1971) found the same difference in number of bacteria in plants of the resistant 'Utah 20' and the susceptible 'Highlander'. The resistance to *C. michiganense* found so far is at least partially based on a diminished multiplication of the pathogen in the plant and does not prevent systemic infection of host tissue.

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