Conditions for symptom development of the syringae leaf spot on tomato seedlings

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Abstract

Pseudomonas syringae pv. syringae, the cause of the syringae leaf spot of tomatoes, incited the development of characteristic dark-brown necrotic spots, 1-2 mm in diameter on 6-week old spray-inoculated or vacuum-infiltrated plants only under mist conditions. A 1 to 3 day post-inoculation moisture period was more critical for the development of the symptoms than a pre-inoculation moisture period. Pre-inoculation wounding with sandbags was necessary for symptom development of spray-inoculated plants and improved symptom development of vacuum infiltrated plants.

Key words: *Tomato, vacuum* infiltration inoculation, spray inoculation, symptom development, *Pseudomonas syringae pv. syringae*

Résumé : Les conditions pour le développement des tâches foliaires de Syringae sur des plantes de tomates

Le développement de taches brunes, nécrotiques (1-2 mm de diamètre) caractéristiques de Pseudomonas syringae pv. syringae (l'agent causal des taches foliaires de la tomate) sur plants de tomate âgés de 6 semaines, est conditionné par une humidité relative élevée. Une période d'humidité de 1 à 3 jours était beaucoup plus critique après l'inoculation qu'avant inoculation pour le développement des symptômes. L'utilisation de petits sacs en tissus de gaze remplis de sable en vue de causer des blessures sur les feuilles était nécessaire avant l'inoculation pour la manifestation des symptômes sur les plants inoculés par pulvérisation. Ces blessures artificielles accentuèrent les symptômes sur plants inoculés par infiltration sous vide.

Mots-clés : Tomate, inoculation par filtration sous vide, inoculation par pulvérisation, développement des symptomes, *Pseudomonas syringae pv. syringae*

ملخص : حالات ظهور التبقع البني لـ«syringae» على أوراق نبات الطماطم.

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Pseudomonas syringae pv.syringae **هو العامل المتسبب في ظهور التبقع البني على أوراق نبات الطماطم. أسفر تطعيم نباتات من الطماطم بلغ سنها ستة أسابيع بطريقتي رش البكتيريا على الأنسجة وارتشاحها بحضور فراغ كامل على ظهور بقع بنية نخرة على الأوراق.**
ظهور هذه الأعراض مرتبط بارتفاع رطوبة الهواء فمدة تتراوح من يوم واحد إلى ثلاثة أيام من الرطوبة العالية بعد عملية التطعيم هي مدة حرجة لظهور الأعراض أكثر من وجود رطوبة قبل التطعيم.
جرح أنسجة أوراق الطماطم باستعمال أكياس صغيرة من الرمل قبل التطعيم بالرش عملية ضرورية لظهور الأعراض ، بينما تحسن من ظهور الأعراض في حالة التطعيم بالارتشاح بحضور فراغ كامل.

الكلمات المفتاحية : طماطم ، تطعيم بالارتشاح بحضور فراغ كامل ، تطعيم بالرش ، ظهور أعراض ،
Pseudomonas syringae pv.syringae

Introduction

Since the late 1970's and early 1980's, *Pseudomonas syringae* pv. *syringae* van Hall has been reported the cause of a tomato (*Lycopersicon esculentum* Mill) leaf spot disease (Gitaitis *et al.* 1985; Jones *et al.* 1981; Wilkie and Dye 1974). The syringae leaf spot is innocuous but in the field it can be confused with other serious leaf-spotting tomato diseases, the bacterial spot and speck caused respectively by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye and *Pseudomonas syringae* pv. *tomato* (Okabe) Young *et al.* (Gitaitis *et al.* 1987; Gitaitis 1991; Jones *et al.* 1983; Jones *et al.* 1986). Furthermore, routine physiological tests usually used to differentiate *Pseudomonas syringae* pv. *tomato* from other fluorescent pseudomonas on tomato (Gitaitis 1991; Jones *et al.* 1981) such as oxidase, arginine dihydrolyse, hypersensitivity reaction on tobacco and soft rot on potato are no more useful. Based on these tests *Pseudomonas syringae* pv. *tomato* and *Pseudomonas syringae* pv. *syringae* are very similar (Gitaitis 1991; Jones *et al.* 1981). Symptoms caused by the two organisms are distinctly different (Gitaitis 1991; Jones *et al.* 1981) and hence a pathogenicity test on tomato could allow a final confirmation. The difficulty to reproduce the syringae leaf spot symptoms in greenhouse has been mentionned (Gitaitis 1991; Jones *et al.* 1981). Information with regard to the most favorable conditions for distincts symptoms of the syringae leaf spot is needed to perform pathogenicity test and hence a correct diagnosis. The purpose of this work was to determine those conditions. Experiments were designed to study the effect of inoculation method, temperature, pre-inoculation host injury, and various types of pre- and post-inoculation moisture on disease development.

Materials and methods

Bacterial strain

A highly virulent strain of *Pseudomonas syringae* pv. *syringae* (designated B-76) was used in all studies. Cultures of the test bacterium was maintained at 6 °C on slants of nutrient yeast dextrose agar (NYDA, 23 g nutrient agar, 5 g yeast extract, 10 g glucose, and 1 l distilled water, pH 6.8) for 6-8 weeks between transfers. Inoculum was produced by streaking plates of King's medium B (King *et al.* 1954) and incubating at 25 °C for 24-48 hr. In the various experiments inoculum concentrations, prepared by washing bacterial growth with sterile distilled water from plate culture grown at 27 °C for 36-48 hr on King's medium B, was determined with a Bausch and Lomb Spectronic 20 set at 590 mm, and the desired concentrations were obtained by appropriate dilutions.

Inoculations

Six-week-old tomato plants (about 15 cm tall) were inoculated by two methods: standard spray inoculation and vacuum infiltration inoculation. In the first method, suspension of 10^8 cfu of the test bacterium per ml was applied to runoff to all plant surfaces with an electric sprayer held about 30 cm from the plant. In the vacuum infiltration procedure, leaves of bare-root tomato plants were infiltrated with a suspension (10^6 cfu/ml) by immersing the foliage and placing plants under a partial vacuum (about 76 cm of Hg) and releasing the vacuum abruptly.

Influence of inoculation procedure and environmental conditions on symptom development

Initial studies with *Pseudomonas syringae* pv. *syringae* suggested that certain conditions, particularly water congestion in tissue, were essential for disease development. Two separate studies were conducted to determine the effect of inoculation method, temperature, and various types of pre-and post-inoculation moisture treatments on disease development. Some treatments involved wounding of leaves, and this was accomplished by rubbing with sterile sandbags. Both spray inoculation and the vacuum infiltration inoculation were used.

In one study, the following treatments were applied to plants of the highly susceptible cultivars FM 6203, Peto 95, and Libby 8990-A, giving a total of 42 treatments combinations:

❒ plants vacuum infiltrated with bacteria, held in the headhouse for 8 hr to reduce the moisture level of foliage, and placed in a 20 °C mist chamber for 3, 4, or 5 days;
❒ plants wounded, vacuum infiltrated, held in the headhouse for 8 hr, and placed in the 20 °C mist chamber for 3, 4, or 5 days
❒ plants sprayed with bacteria and placed in the mist chamber for 3, 4, or 5 days;
❒ plants wounded, sprayed with bacteria, and placed in the mist chamber for 3, 4, or 5 days;

❒ plants wounded, vacuum infiltrated with sterile distilled water, held in the headhouse for 8 hr, and placed in the mist chamber for 2 days (control) ;
❒ plants wounded, sprayed with sterile distilled water, and placed in the mist chamber for 2 days (control).

Five replications of each bacterial treatment and ten replications of the two controls were arranged in a completely randomized design in the mist chamber and in a 20 °C growth chamber after the mist treatment. Plants were observed daily for evidence of disease, and lesion counts were made 10-12 days after inoculation.

In a related study the following treatments were applied to plants of the FM 6203 tomato cultivar, and the plants receiving each treatment were placed in growth chambers both at 20 and 25 °C.

❒ plants placed in mist for 24 hr to induce water congestion in leaves, then vacuum infiltrated or sprayed with inoculum, and placed in mist for 1, 2, or 3 days;
❒ plants placed in mist for 24 hr, wounded, and then vacuum infiltrated or sprayed and placed in mist for 1, 2 or 3 days;
❒ plants taken directly from the greenhouse and vacuum infiltrated or sprayed, and placed in mist for 1, 2 or 3 days;
❒ plants taken from greenhouse, wounded, vacuum infiltrated or sprayed, and placed in mist for 1, 2 or 3 days;

❒ plants taken from the mist and the greenhouse, wounded, sprayed or infiltrated with sterile distilled water, and placed in mist for 2 days (control).

All vacuum infiltrated plants were held for 8 hr in the headhouse to reduce water congestion in foliage before being placed in the mist chamber. After receiving the above treatments, five replications of each treatment combination (total of 28 at each temperature) were arranged in a completely randomized design in growth chambers at 20 and 25 °C. Plants were observed daily for evidence of disease, and lesion counts were made 10-12 days after inoculation.

Results

Lesions developed on both wounded and non wounded Peto 95, Libby 8990-A and FM 6203 plants that were infiltrated with *Pseudomonas syringae* pv. *syringae* and placed under continuous mist (Tables 1 and 2). Lesion counts did not differ significantly (P=0,05) among the three cultivars although FM 6203 appeared to be slightly more susceptible (Table 1). Symptoms developed on plants regardless of the duration of the mist period after infiltration (Tables 1 and 2). Lesion counts were sometimes higher when plants were wounded, but results were inconsistent. Few lesions developed when plants were water-soaked (placed under continuous mist) before infiltration (Table 2). Lesions developed on spray-inoculated plants only if they were wounded, water-soaked, or water-soaked and wounded before inoculation (Tables 1 and 2). Significantly, more lesions developed on spray-inoculated plants that were wounded than on those water-soaked (Table 2). Except when plants were water-soaked or water-soaked and wounded, lesion counts were always significantly higher when plants were vacuum infiltrated than when spray-inoculated, although the inoculum concentration in the latter case

was 100 times higher. Lesion counts on both vacuum infiltrated and sprayed plants were similar at 20 and 25 °C (Table 2).

Table 1. Influence of inoculation procedure, various periods of post-inoculation mist, and wounding on disease development on three highly susceptible tomato cultivars inoculated with *Pseudomonas syringae pv.syringae* at 20 °C

Cultivar	Pre-inoculation treatment	Post-inoculation mist (days)	Mean number of lesion on plants inoculated by two methods[a]	
			VIP[b]	SSI[c]
Peto 95	Not wounded	3	55*	0
		4	65*	0
		5	70*	0
	Wounded[d]	3	80*	51
		4	85*	55
		5	75*	55
Libby 8990-A	Not wounded	3	66*	0
		4	80*	0
		5	85*	0
	Wounded	3	90*	55
		4	85*	60
		5	90*	62
FM 6203	Not wounded	3	72*	0
		4	80*	0
		5	85*	0
	Wounded	3	103*	60
		4	112*	65
		5	102*	65
		FLSD (p = 0.05)	20	12

[a] Each value is an average of five replications. Lesion counts were made 10-12 days after inoculation
[b] VIP = vacuum infiltration procedure; plants were infiltrated with a suspension containing 10^6 cfu/ml
[c] SSI = standard spray inoculation; plants were sprayed to runoff with a suspension containing 10^8 cfu/ml
[d] Plants were rubbed with sterile sandbgs
[e] Asterisk indicates that the value is significantly greater than the value for the spray inoculation for the same cultivar and with the same pre-and post-inoculation treatments, as determined by a t-test comparison (P = 0,05)

Table 2. Influence of inoculation procedure and various pre-and post-inoculation treatmnents on disease development on FM 6203 tomato plants inoculated with *Pseudomonas syringae pv. syringae* and maintained at two temperatures

Cultivar	Pre-inoculation treatment	Post-inoculation mist (days)	Mean number of lesion on plants inoculated by two methods[a]	
			VIP[b]	SSI[c]
20	None	1	140*	0
		2	130*	0
		3	210*	0
	Water-soaked	1	0	24
		2	1	32
		3	1	28
	Wounded	1	140*	57
		2	200*	56
		3	160*	65
	Water-soaked and wounded	1	90	70
		2	65	90
		3	43	80
25	None	1	200*	0
		2	120*	0
		3	170*	0
	Water-soaked	1	1	22
		2	1	25
		3	0	20
	Wounded	1	330*	62
		2	240*	46
		3	230*	36
	Water-soaked	1	115	70
		2	70	77
		3	66	85
		FLSD (P = 0,05)	45	17

[a] Each value is an average of five replications. Lesion counts were taken 10-12 days after inoculation

[b] Water-soaked or congestion of foliage was created by placing plants in light continuous mist during a 24-hr period before inoculation. Wounding was achieved by rubbing both leaf surfaces lightly with sterile sandbags

[c] VIP = vacuum infiltration procedure; plants were infiltrated with a suspension containing 10^6 cfu/ml

[d] SSI = standard spray inoculation; plants were sprayed to runoff with a suspension containing 10^8 cfu/ml

[e] Asterisk indicates that the value is significantly greater than the value for the spray inoculation at the same temperature, pre- and post-inoculation treatments, as determined by a t-test comparison (P = 0,05)

Discussion

Temperature does not seem to be as critical for the development of lesions caused by *Pseudomonas syringae* pv. *syringae* as for *Pseudomonas syringae* pv. *tomato* (Schneider and Grogan 1978; Smitley and McCarter 1982) and *Xanthomonas campestris* pv. *vesicatoria* (Basu 1966). Similar numbers of lesions developed on plants vacuum infiltrated and placed at 20 and 25 °C. Moisture was the most critical factor in the development of lesions caused by *Pseudomonas syringae* pv. *syringae* regardless of whether plants were vacuum infiltrated or inoculated by spraying. In initial studies, when vacuum infiltrated plants were placed directly in the growth chamber without a moisture treatment, few lesions developed regardless of the cultivar used. In later studies, vacuum infiltrated plants placed in light and continuous mist for 1 to 3 days after infiltration developed dark-brown necrotic spots, 1-2 mm in diameter, that were typical of those observed in the field (Jones *et al.* 1981). Longer periods of mist did not result in increased lesion development as plants deteriorated because of the high moisture stress. Also, lesion counts given the 24-hr mist period before vacuum infiltration were lower than when plants were given only the post-infiltration moisture treatments. Apparently the 24-hr pre-treatment results in water congestion in leaves, which reduces the quantity of the test solution that actually penetrates. Plants that had their leaves wounded with sandbags in-addition to receiving the pre-infiltration mist period before vacuum infiltration had higher lesion counts than plants that received only the mist period. Wounding, either by mechanical means or by water congestion, apparently is necessary when low levels of the bacterium are present. For example, plants of susceptible cultivars sprayed with normal inoculum levels (10^8 cfu/ml) did not develop lesions unless they were either wounded with sangbags or subjected to very high moisture levels before and after inoculation. Under optimum conditions, lesion counts were consistently higher when plants were vacuum infiltrated with suspensions containing 10^6 cfu/ml than when they were spray inoculated with suspensions containing 10^8 cfu/ml. Apparently, the vacuum infiltration procedure is effective in placing cell in highly suitable infection court.

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