Effects of Watersoaking on Response to Xanthomonas vesicatoria in Pepper Leaves

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ABSTRACT

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Detached pepper leaves watersoaked after infiltration with *Xanthomonas vesicatoria* (XV) did not develop a hypersensitive response (HR). Bacterial concentration in the inoculum and multiplication in vivo during incubation resulted in bacterial populations in excess of the minimal concentration required to induce HR, but only a small increase in electrolyte leakage was detected after 24-36 hr of incubation. Typical development of HR began 2-3 hr after watersoaking was permitted to dissipate from leaves kept watersoaked for various periods ranging from 0-6 hr after inoculation. Patterns of bacterial multiplication and electrolyte leakage in detached leaves kept watersoaked for 72 hr after infiltration inoculation with either of two pathotypes of XV were similar to results from susceptible leaves left in situ after inoculation.

Additional key words: *Capsicum annuum*, bacterial leaf spot of pepper, bacterial multiplication, electrolyte leakage.

Inoculation of leaves with phytopathogenic bacteria by the infiltration technique described by Klement (8) has permitted critical study of plant-bacterium interactions. The water in which bacteria usually have been suspended for inoculation is quickly dissipated with no apparent influence on the introduced bacteria or development of host response. Hypersensitivity to infiltration with either pseudomonad or xanthomonad bacteria is characterized by rapid increase in loss of electrolytes from inoculated leaves, a precipitous decrease in bacterial population in vivo, and early development of necrosis (2, 9). A less-dramatic form of hypersensitivity has been described (1) for one pathotype of *Xanthomonas vesicatoria* (Doidge) Dows. (3). Infection of susceptible host tissue with pathogenic bacteria results in a gradual increase in electrolyte leakage accompanied by continual increase in bacterial population in vivo until development of necrosis (2, 12).

The rapidity of related events in development of hypersensitivity on inoculated leaves in situ has prevented accurate determination of the chronology of the associated phenomena. Leaves detached immediately after infiltration remain watersoaked for an extended period and development of the hypersensitive response is delayed. This observation prompted study of the influence of watersoaking on development of hypersensitivity by pathotypes of *X. vesicatoria* in leaves of pepper (*Capsicum annuum* L.) cultivars.

MATERIALS AND METHODS

Leaves of pepper cultivars 10-R and 23-1, which is homozygous for hypersensitive response (HR) to pepper strain, race 2 of *X. vesicatoria* (XV) and cultivar Yolo Y, which is hypersensitive to the tomato strain of XV, were inoculated by infiltration. Bacteria from 24-hr agitated nutrient broth cultures were harvested by centrifugation and the pelleted cells were resuspended in water to 10^8 cells/ml as determined photometrically (50% transmission at 625 nm). While still watersoaked, leaves were detached, the severed petioles immersed in tap water and the laminae enclosed in plastic bags. Laminae of control leaves were left uncovered. In one experiment, whole plants in individual 10-cm diameter clay pots were placed in plastic bags to keep inoculated leaves watersoaked.

Bacterial multiplication and progress of HR in inoculated leaves were followed by periodic determination of electrolyte leakage and bacterial concentration in vivo.

Samples of inoculated leaf tissue, taken as disks cut with cork borers, were assayed to determine bacterial populations in vivo (50 mm^2 total leaf tissue/replicate) and electrolyte leakage (19.24 cm^2 total leaf tissue/replicate). Populations of bacteria in vivo were determined by triturating tissue samples in 1 ml sterile water or 0.85% saline from which tenfold dilutions were made and aliquots of the resulting suspensions were seeded on sterile plates of nutrient agar. Numbers of bacterial colonies that appeared after incubation of the seeded plates for 3 days at 30 C were considered indicative of the bacterial concentration in vivo at time of sampling. Tissue samples for electrolyte leakage determinations were each placed in 12 ml of distilled water and electrical conductivity of the water measured immediately and again after 1 hr on a horizontal shaker [120 strokes (4 cm) per minute]. Increase in the two readings (μmhos) was interpreted as an indication of damage to the host cell membrane system attributable to bacteria.

TABLE 1. Effect of extended watersoaking on electrolyte loss from pepper leaves (cultivar 10-R) infiltrated with pepper strain (race 2) of *Xanthomonas vesicatoria*

Incubation time following removal of plastic bags (hr)	Conductivity (μmhos)[a] Period of continuous watersoaking (hr)[b]						
	0	1	2	3	4	5	6
0	24	42	30	31	39	29	33
1	35	29	34	33	21	24	32
2	21	29	58	115	59	32	115
3	78	138	292	310	142	389	409
4	278	422	508	615	504	508	500

[a]Each figure (μmhos) is average of two replicates.
[b]Whole plants were placed in plastic bags to maintain watersoaking of inoculated leaves.

TABLE 2. Influence of watersoaking on electrolyte loss and bacterial multiplication in detached pepper leaves (cultivar 23-1) inoculated with pepper strain (race 2) of *Xanthomonas vesicatoria*

Incubation (hr)	Duration of watersoaking	
	Continuous	Temporary
	Conductivity (μmhos)	
0	35[b]	35[b]
16	30	34
24	33	214
40	57	352
	In vivo bacterial concentration ($\times 10^6$)	
0	2.1[b]	2.1[b]
16	4.7	4.3
24	6.4	2.8
40	5.7	1.3

[a]Leaves incubated on laboratory bench at approximately 25 C.
[b]All figures represent averages of 15 replicated treatments indicated; 0 hour data are averages of the same 15 replicates.

RESULTS

No electrolyte loss was detected from inoculated leaves maintained in a watersoaked condition for up to 6 hr (Table 1). Electrolyte leakage was first noted 2-3 hr after plastic bags were removed from inoculated leaves maintained in a watersoaked condition for 0-6 hr. Watersoaking disappeared within 30 min after the plastic bags were removed. Progress of HR, as determined by first detectable increase in electrolyte loss and eventual maximum electrolyte leakage after watersoaking dissipated, was strikingly similar for all treatments.

Progress of HR in detached leaves (increase in electrolyte leakage and decrease in bacterial population) was not apparent until after 16 hr of incubation in leaves that were only temporarily watersoaked (not covered with plastic bags) (Table 2). Essentially no increase in electrolyte leakage was noted in some experiments after 40 hr in leaves kept watersoaked although bacterial populations in vivo more than trebled in 24 hr and remained more than 2.5 times the original concentration after 40 hr. No increase in electrolyte loss but considerable bacterial multiplication was detected in leaves infiltrated with the tomato strain of XV (Table 3) and maintained watersoaked for 48 hr. These results were in distinct contrast to bacterial multiplication and electrolyte loss in detached leaves temporarily watersoaked (left uncovered after inoculation) and in leaves left in situ (attached to the plant). Increase in bacterial concentration in vivo in leaves kept watersoaked was considerably greater than in inoculated leaves temporarily watersoaked and detached or permitted to remain in situ.

Increases in bacterial concentration in vivo and electrolyte loss during extended incubation in a watersoaked condition were determined in detached leaves of a single pepper cultivar inoculated separately with two bacterial isolates that characteristically induce hypersensitive and susceptible responses (Table 4). Maximum electrolyte loss after 72 hr of incubation was somewhat greater in the hypersensitive bacterium-host combination and was attributed to a slightly higher concentration of bacteria in vivo. Persistent watersoaking in the absence of pathogenic bacteria caused no detectable change in leakage of electrolytes from host tissues during the 72-hr incubation.

DISCUSSION

In these experiments hypersensitivity of the host was not dependent strictly on in vivo concentration of the bacterial pathogen. In all the studies the concentration of bacterial cells introduced at inoculation was sufficient to induce the HR (10). Bacterial multiplication continued, for the most part, throughout the entire incubation period in leaves maintained in watersoaked condition. Final concentrations of bacteria were well above those in leaves that remained in situ or were only temporarily watersoaked. That hypersensitivity did not result from these bacterial concentrations was further verified by absence of rapid increase in electrolyte loss (2, 5); this was interpreted as evidence that the host cell membrane system remained essentially intact except under conditions of prolonged incubation (more than 36-48 hr). The patterns of electrolyte loss and bacterial multiplication in vivo in leaves infiltrated with a suspension of either of two pathotypes of the bacterium and maintained in a watersoaked condition for an extended incubation period resembled those of typical susceptibility in inoculated leaves temporarily watersoaked and left in situ (12). It was concluded from these studies that persistent watersoaking prevented, or blocked, development of HR as suggested previously (13). The nature or cause of this action was not

TABLE 3. Influence of watersoaking on electrolyte loss and bacterial multiplication in pepper leaves (cultivar Yolo Y) inoculated with the tomato strain of *Xanthomonas vesicatoria*

Incubation (hr)	Detached leaves[a] with watersoaking		In situ leaves[a] with temporary watersoaking
	Continuous	Temporary	
	Conductivity (μmhos)		
0	34	35	36
24	30	32	53
40	42	67	101
48	52	86	130
	In vivo bacterial concentration ($\times 10^6$)		
0	3.1	3.1	3.1
24	9.9	7.5	3.1
40	25.9	10.2	7.6
48	29.9	9.0	16.4

[a]Detached leaves incubated on laboratory bench at approximately 25 C; in situ leaves incubated in a growth room at 30 C.
[b]All figures represent averages of 18 replicated treatments; 0-hr data include measurements from the same 12 replicates.

TABLE 4. Electrolyte loss and bacterial multiplication in vivo in leaves of cultivar 10-R pepper kept watersoaked for 72 hr after infiltration inoculation with isolates 69-20 (hypersensitive response) and 68-1 (susceptible response) of *Xanthomonas vesicatoria*

Inoculum applied	Incubation time (hr)					
	0	24	36	48	60	72
	Conductivity (μmhos)					
H_2O (control)	27[a]	36	41	29	35	47
69-20	27	77	99	162	242	386
68-1	34	39	70	124	196	236
	Bacterial concentration in vivo ($\times 10^6$)					
69-20	0.4	6.0	47	29	74	...
68-1	0.9	1.2	25	23	63	52

[a]Each figure is average of three replicates.

determined although other conditions have been reported to alter response to (bacterial) infection (6, 7, 11). Continuous flooding of the intercellular spaces may have prevented attachment of the bacteria to the host cells which has been suggested as essential for bacterial pathogenesis (4). Prevention of HR did not preclude host response indistinguishable from typical susceptibility. These results were considered substantiating evidence that HR and susceptible host response to XV infection in pepper result from distinct biological processes.

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