Characterization of Hypersensitivity in Capsicum annuum Induced by the Tomato Strain of Xanthomonas vesicatoria

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ABSTRACT

The hypersensitive response induced in two cultivars of *Capsicum annuum* by isolates of the tomato strain of *Xanthomonas vesicatoria* were characterized according to several criteria. Response to inoculation in leaves of both pepper cultivars was not visibly evident until after ca. 15-hr incubation at 30 C and 24 hr at 25 C. An initial decrease in bacterial concentration in vivo was found after 6-hr incubation at the higher temperature, but this was followed by a progressive increase in concentration for the next 18 hr. Electrolyte-loss patterns from both pepper cultivars intermediate to those induced by pepper strain, race 2 of the bacterium were induced by six isolates of the tomato organism. Although electrolyte losses from both pepper types induced by tomato isolate 71-14 were consistently lower than for five other isolates of the tomato pathotype, they generally followed the same pattern. Incubation at 25 C, rather than 30 C, retarded electrolyte loss from leaves inoculated with the tomato strain of the bacterium but incubation of leaves in darkness, as opposed to light, enhanced electrolyte loss at both temperatures. Introduction of bacterial cells suspended in 0.15 N $Ca(NO_3)_2$ instead of sterile distilled water also enhanced electrolyte loss, more in one pepper cultivar than in the other. These results provide evidence that distinct forms of hypersensitivity may be induced in pepper by inoculation with different pathotypes of *X. vesicatoria*.

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Additional key word: light.

Hypodermic infiltration of leaves with suspensions of bacteria as described by Klement (6) provides a convenient and reliable method for assaying pathogenic capabilities of isolates (7). Many phytopathogenic bacteria induced a hypersensitive reaction (12) when inoculated into plants not susceptible to infection in nature (8). Various factors, including concentration of cells and viability of the bacteria (9), calcium (3), light, and pre-inoculation treatment of host tissues (5, 11), have been shown to influence development of the hypersensitive response.

Pathotypes of phytopathogenic bacteria species have been distinguished according to host range, type of symptom induced, and incubation time prerequisite for symptom development (2, 10). Response to one pathotype of *Xanthomonas vesicatoria* (Doidge) Dows. (race 2, pepper strain), that resulted in hypersensitivity on some pepper (*Capsicum annuum* L.) types, was found to be simply inherited and dominant (1, 12, 14).

Obvious differences in symptom development patterns and appearance of affected tissues in hypersensitive responses have been noted, but classified only as hypersensitive or susceptible. Recognition of distinctive types of hypersensitive responses induced in pepper by pathotypes of *X. vesicatoria* prompted this study of symptoms caused by the tomato strain.

MATERIALS AND METHODS.—Two isolates of *X. vesicatoria* originally isolated from naturally infected peppers and tomatoes grown in Florida and identified as E-3 (pepper strain, race 2) and 71-14 (tomato strain) were used throughout these studies. Five other isolates of the tomato strain isolated by R. E. Stall from infected plants grown in Florida and identified as 67-11, 67-21, 68-8, 70-2, and 71-32 were used also.

Inoculum was prepared with cells from 24-hr nutrient broth shake cultures centrifuged 10 min at 1,500 *g*, resuspended in sterile distilled water and diluted appropriately to give 50% transmittance (625 nm) photometrically; i.e., 10^8 cells/ml. Where indicated, bacterial cells were suspended in 0.15 N $Ca(NO_3)_2$.

Plants of two pepper cultivars were used exclusively. A breeding line designated 23-1-7 (23-1), homozygous for hypersensitive response to pepper strain, race 2 of *X. vesicatoria* and 'Yolo Y' (YY), a cultivar homozygous for susceptibility to this bacterial isolate. Individual test plants were grown in the greenhouse in 15-cm pots until some leaves were nearly full size and then moved to temperature-controlled (±2 degrees) growth rooms in which all experiments were conducted. Light 6430 lux (ca. 600 ft-c) was supplied for all plants from "Cool-white" and "Gro-lux" fluorescent tubes placed approximately 45 cm above the plants. "Intermittent light" is used herein to designate 16-hr exposure to light followed by 8-hr darkness.

All inoculations were accomplished by hypodermic infiltration of leaves. In vivo bacterial multiplication was determined by counting colonies on plates incubated at 30 C after seeding with aliquots of appropriate dilutions from 50 mm^2 inoculated leaf tissue triturated in sterile distilled water. Electrolyte loss was measured as the increase in conductance of the water (20 ml sterile distilled) in which was suspended fifteen 16-mm diam disks of inoculated leaf tissue vacuum infiltrated for 2 min prior to shaking (100 3.75-cm cycles/min) for 1 hr.

RESULTS.—Leaves of both 23-1 and YY plants incubated at 30 C did not exhibit visible symptoms until ca. 15 hr after inoculation with isolate 71-14. The first symptom was loss of turgor which became progressively more severe and ultimately resulted in complete necrosis characterized by uneven collapse

and coloration of inoculated areas. Tissues killed by isolate 71-14 (the tomato strain) remained substantially darker than tissues killed by the pepper strain, race 2 of *X. vesicatoria* (14). Differences in symptom development in 23-1 and YY peppers were not readily evident visually, although some differences in electrlyte loss were recorded (Fig. 1).

Five other isolates of the tomato strain of *X.*

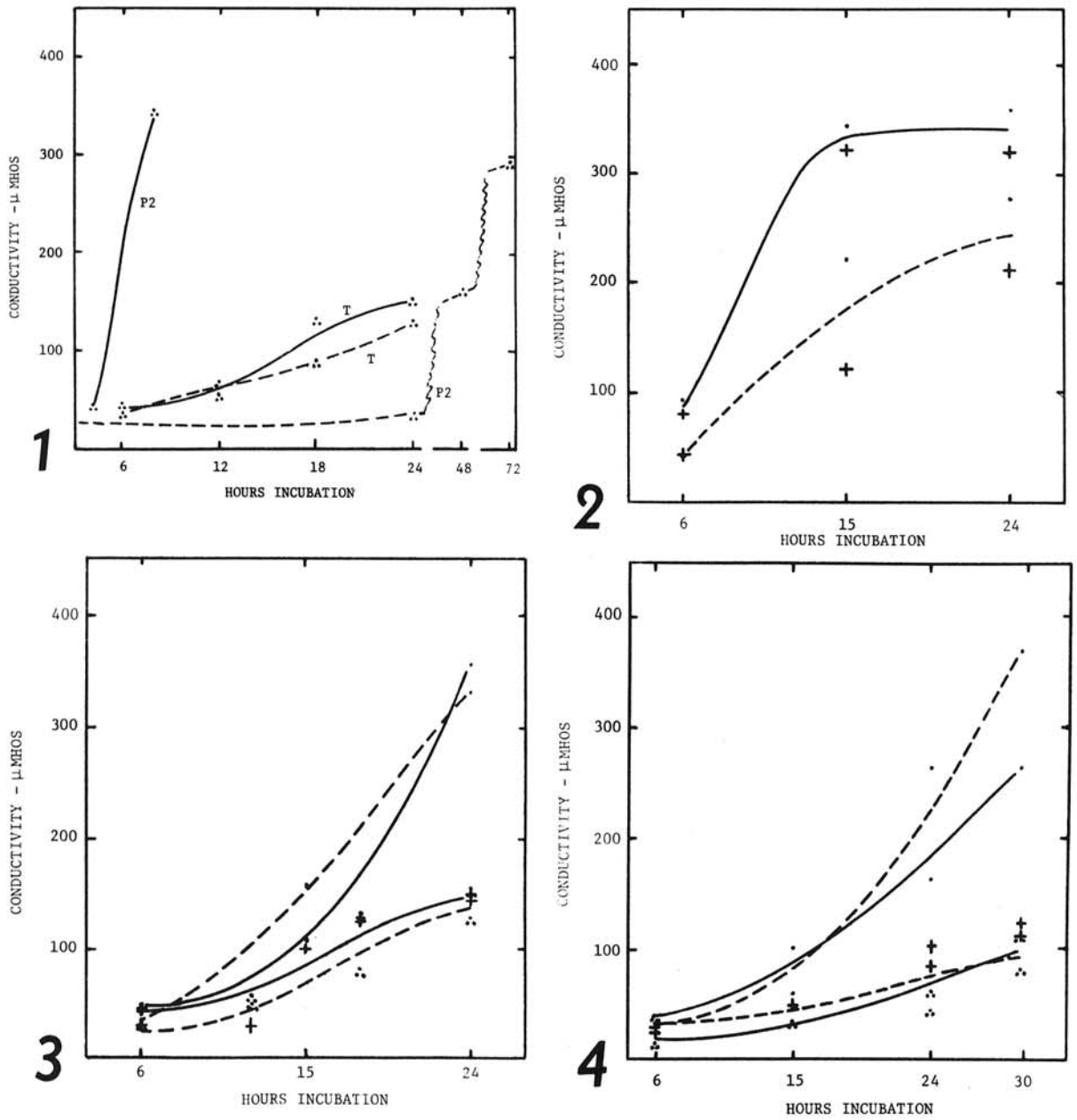

Fig. 1-4. Average electrolyte losses from leaves of 23-1 (solid lines) and YY (broken lines) pepper incubated in continuous light at 30 C after being inoculated with pepper strain, race 2 (P2) (three replicates) and tomato strain, isolate 71-14 (T) (9 to 12 replicates) of *Xanthomonas vesicatoria*. **2)** Average electrolyte losses from leaves of 23-1 (solid line) and YY (broken line) peppers incubated in intermittent light (+) (36 replicates) and continuous darkness. (.) (18 replicates) at 30 C after being inoculated with five tomato strain isolates of *X. vesicatoria*. **3)** Comparison of average electrolyte losses (6 to 12 replicates each) from leaves of 23-1 (solid lines) and YY (broken lines) peppers inoculated with tomato strain isolate 71-14 of *X. vesicatoria* and incubated in complete darkness (.) versus intermittent (+) or continuous (∴) light at 30 C. **4)** Comparison of average electrolyte losses (6 to 12 replicates each) from leaves of 23-1 (solid lines) and YY (broken lines) peppers inoculated with tomato strain isolate 71-14 of *X. vesicatoria* and incubated in complete darkness (.) versus intermittent (+) or continuous (∴) light at 25 C.

vesicatoria were tested on the same plants. Electrolyte losses were somewhat greater from both pepper types than with isolate 71-14 (Fig. 2), but the patterns of electrolyte loss in the two peppers were consistently different (intermediate) from those established for pepper strain, race 2 isolates of the bacterium (Fig. 1). Greater differences in electrolyte loss from the two pepper types were obtained with the other tomato strain isolates than with 71-14, however.

Multiplication in vivo of isolate 71-14 was followed in both pepper cultivars during incubation in intermittent light at 30 C (Table 1). Similar patterns of bacterial concentrations in vivo were obtained for both plant types wherein there was a slight reduction in bacterial population after 6 hr incubation, followed by continual increase for the next 18 hr.

Electrolyte loss from leaves of both pepper types inoculated with isolate 71-14 was found to be influenced in similar manner by temperature and light regimes during incubation (Fig. 3, 4). The effect of temperature was most noticeable after 24 - hr incubation, particularly when plants were exposed to light after inoculation. Conductivity readings from plants held at 30 C were approximately twice those from plants held at 25 C for this incubation period if exposed to light after inoculation. Highest electrolyte losses at both temperatures were obtained from plants maintained in darkness after inoculation, but again, losses were substantially greater at 30 C. No consistent differences were noted in the responses of the two pepper cultivars in these experiments.

Electrolyte loss from leaves of both pepper types was determined following inoculation with bacterial cells suspended in 0.15 N $Ca(NO_3)_2$. Leaves on the same plants inoculated at the same time with bacterial cells from identical cultures suspended in sterile distilled water were used as controls. Electrolyte loss from leaves of both plants injected with bacterial cells of 71-14 suspended in calcium solution was enhanced (Fig. 5). The effect on electrolyte loss was greater with YY leaves than the 23-1 leaves. These results were in contrast to reduced electrolyte loss from 23-1 leaves inoculated with bacterial cells of pepper strain, race 2 of *X. vesicatoria* suspended in a similar calcium solution.

DISCUSSION.—Attention to disease-inducing capabilities of phytopathogenic bacteria has been directed largely to distinction of susceptible vs. hypersensitive plant responses. While differences in hypersensitive reactions to bacterial inoculations have been noted, and some taxonomic significance attached (10), little attention has been given to characterization of these "variant" plant responses. In earlier studies we noted that the pathotype designated as the tomato strain of *X. vesicatoria* consistently induced a visibly different kind of hypersensitive response on (all) pepper plants than that caused by isolates of the pepper strain, race 2 on selected pepper cultivars (2). Such differences were not related to concentrations of inocula (5).

That the response induced in pepper by the tomato strain of *X. vesicatoria* may be properly termed "hypersensitivity", is supported by development of visible symptoms in 24 hr as opposed to 48 hr or more required for appearance of susceptible symptoms (12). Distinct variations in ability to induce electrolyte loss were noted among isolates of the tomato strain. However, the patterns of electrolyte loss for these bacterial isolates on both pepper cultivars tested were definitely intermediate to those previously established for bacterial isolates of pepper strain, race 2, on similar pepper types (1).

In contrast to results obtained with the pepper strain, race 2, light was found to consistently retard electrolyte loss from pepper leaf tissues inoculated with the tomato strain of the bacterium. More severe symptom development on plants incubated in

TABLE 1. Numbers of colonies ($\times 10^5$) of isolate 71-14 of the tomato strain of *Xanthomonas vesicatoria*, recovered after the indicated incubation under intermittent light at 30 C in leaves of two pepper cultivars infiltrated with 10^8/ml bacteria

Pepper Cultivar	Hours of incubation				
	1	6	12	18	24
23-1-7[a]	1.7	1.1	2.4	3.4	4.9
Yolo Y[b]	2.1	1.0	3.6	4.4	5.7

[a]Average of nine replicates.
[b]Average of six replicates.

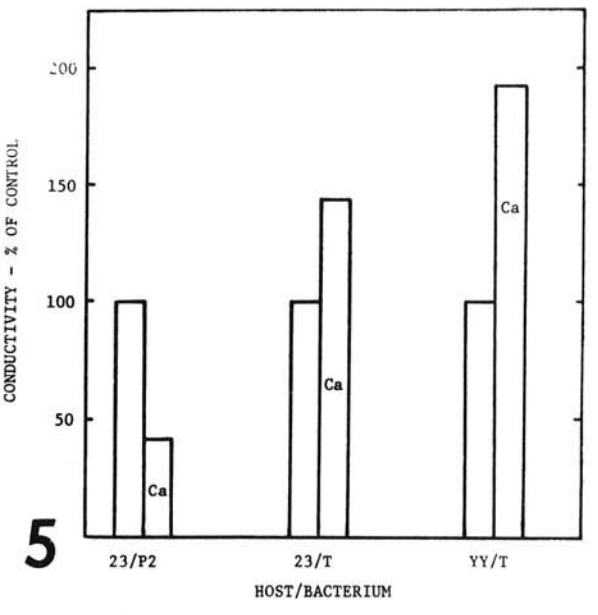

Fig. 5. Electrolyte losses (averages of 12 replicates) induced in leaves of 23-1 (23) and YY peppers inoculated with pepper strain, race 2 (P2), and tomato strain isolate 71-14 (T) of *Xanthomonas vesicatoria* suspended in sterile distilled H_2O (control = 100%) or 0.15 N $Ca(NO_3)_2$ (Ca).

darkness than in light, has been reported previously for xanthomonad bacteria (5). Incubation with reduced air temperature also served to retard electrolyte loss, but effect of temperature was less influential than light, as evidenced by the enhanced electrolyte loss at 25 C in darkness.

Earlier studies had provided evidence that rapid deterioration of the cell membrane system characterized the hypersensitive reaction induced in pepper by *X. vesicatoria* (13). This hypothesis was supported by preliminary histological examinations of inoculated leaf tissues and, further, by reduction of electrolyte loss when inoculum was amended with calcium solutions or calcium was used as a pre-inoculation treatment. It has been reported that calcium is associated with cell membrane integrity (4). These observations and results were in general agreement that deleterious effects on the selective permeability of the cellular membrane system are often an early result of infection by phytopathogens (15). The determination that calcium enhances electrolyte loss from pepper leaves inoculated with the tomato strain of *X vesicatoria* is additional evidence that hypersensitivity induced by distinct pathotypes may involve quite different biological processes (5).

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