Different Phenotypes Associated with Incompatible Races and Resistance Genes in Bacterial Spot Disease of Pepper

A. M. HIBBERD, Former Graduate Assistant, Vegetable Crops Department, R. E. STALL, Professor, Plant Pathology Department, and M. J. BASSETT, Professor, Vegetable Crops Department, University of Florida, Gainesville 32611

ABSTRACT

Hibberd, A. M., Stall, R. E., and Bassett, M. J. 1987. Different phenotypes associated with incompatible races and resistance genes in bacterial spot disease of pepper. Plant Disease 71:1075-1078.

Three distinct resistance systems to *Xanthomonas campestris* pv. *vesicatoria* are distributed among plants of PI 271322 of *Capsicum annuum*. One plant was selected from a population of PI 271322 that was homozygous for all three resistances, and progeny of the plant were designated line 271-4. Multiplication of a strain of each incompatible race in leaves of this line ceased at different population levels. The time to host cell collapse (phenotype) after inoculation with 5×10^8 cfu/ml was different for each resistance and incompatible race. The phenotypes and multiplication of incompatible strains in heterozygotes differed from that in homozygotes. These resistances may be incompletely dominant.

Three single genes in pepper (*Capsicum annuum* L.) have been reported to confer resistance to bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye (*X. c.* pv. *vesicatoria*). These genes have been designated *Bs*1, *Bs*2, and *Bs*3 and were first found in PI 163192, PI 260435, and PI 271322, respectively (1,3,10). The genes control hypersensitive reactions (HR) to certain strains of *X. c.* pv. *vesicatoria*. The HR associated with these genes can be recognized after infiltration of leaves with concentrated inoculum (10^8 cfu/ml) of incompatible strains. However, the HR has been confirmed with only two of the genes, *Bs*1 and *Bs*2, by determining electrolyte leakage patterns and bacterial multiplication in inoculated leaves (2,4,12).

The *Bs*1 gene was reported in 1963 but was never used commercially, because a race of the pathogen (race 1) existed in the field that was pathogenic to breeding lines with this resistance gene. Strains of race 2 induce HR in plants with *Bs*1, but strains of race 1 do not (5). Race 2 is the most common race in Florida, but race 1 predominates in other pepper production areas of the world (6).

Present address of first author: Department of Primary Industries, P.O. Box 327, Cleveland, Queensland, Australia 4163.

Florida Agricultural Experiment Station Journal Series No. 8103.

Accepted for publication 7 July 1987 (submitted for electronic processing).

The *Bs*3 gene was selected in plants of PI 271322 by screening for resistance to strains of race 1. However, in addition to the resistance to strains of race 1, two other resistances also were detected in plants of PI 271322. One had the same pathogen specificity as the *Bs*1 gene and was allelic with *Bs*1 in testcrosses (9). The other resistance was best selected by quantitatively assessed variables (lesion numbers per unit area of leaf and lesion diameters) after infiltration of leaves with low concentrations of inoculum (10^3–10^4 cfu/ml). Inheritance of this resistance conformed to that expected for two recessive genes and was effective against all strains of *X. c.* pv. *vesicatoria* that were tested (11).

The purpose of this paper is to report that each of the three resistances in progeny of a single-plant selection of PI 271322 has a distinctive phenotype when inoculated with an incompatible race of *X. c.* pv. *vesicatoria*.

MATERIALS AND METHODS

Host material. Seeds of PI 271322 of *C. annuum* were obtained from the South Atlantic Regional Plant Introduction Station, Experiment, GA. Seeds of Early Calwonder (ECW) and Early Calwonder-10R (ECW-10R), a near-isogenic line with the *Bs*1 gene, were on hand. Line 271-4, which contained *Bs*1, *Bs*3, and the quantitatively assessed resistance, was selected from PI 271322, and seeds were obtained from a single plant. Seeds of reciprocal hybrids were obtained by crossing the resistant parent, 271-4, with ECW. Both 271-4 and ECW served as female parents in the crosses, and the seeds from each female parent were kept separate.

Plants were raised in a steamed peat-vermiculite mix in 10-cm pots arranged in a greenhouse. The temperature in the greenhouse ranged from 20 to 35 C. Plants were watered as required and treated four times during experiments with about 0.4 g/pot of soluble 20:20:20 fertilizer.

Inoculum preparation. The *X. c.* pv. *vesicatoria* strains used were isolated from pepper plants in the field in Florida and stored in sterilized tap water. Strains also were maintained in 15% glycerol at −20 C for short-term storage between tests. Inocula were prepared from nutrient broth cultures that had been shaken for 24 hr and then centrifuged at $1{,}000 \times g$. The harvested bacterial cells were then suspended in sterile tap water and diluted to an absorbance of 0.3 at a wavelength of 600 nm (= 5×10^8 cfu/ml). This concentration was used directly or diluted to 5×10^3 cfu/ml before inoculation of leaves by hypodermic infiltration of intercostal leaf tissues.

Selection of race three. Leaves of 166 plants of PI 271322 were inoculated with 16 strains of *X. c.* pv. *vesicatoria*, each adjusted to 5×10^8 cfu/ml. The plants were arranged in randomized rows of seven or eight plants per row on a greenhouse bench. Leaves below the first fork were inoculated. Each of four strains was infiltrated into an area of 4–5 cm^2 of a leaf, and four mature leaves on each plant were used to test all 16 strains. Disease reactions were compared with those on plants of ECW and ECW-10R inoculated with the same strains of *X. c.* pv. *vesicatoria* and in the same manner.

Bacterial populations. The resistance in plants of 271-4 to strains of the three races was more clearly defined by determinations of population dynamics of the strains in leaves. In addition, the strains were inoculated into leaves of the susceptible cultivar, ECW, and a strain of race 2 was inoculated into leaves of ECW-10R for an HR control.

The numbers of cells in leaves were determined at timed intervals after infiltration of 4–5 cm^2 of leaf tissue with about 5×10^3 cfu/ml. At each sampling,

two disks (0.5 cm^2 each) of inoculated tissue were triturated in 0.5 ml of sterile tap water. The resulting suspensions were serially diluted where appropriate, 0.05 ml of the final dilutions was spread onto nutrient agar plates, and the plates were incubated at 30 C for 2–3 days. The numbers of colonies were transformed to $\log_{10}$ values. The experiment was repeated, and each experiment had three replicates. The results of only one experiment are reported.

Electrolyte leakage. The period of time between inoculation and necrosis was determined from electrolyte leakage patterns. A strain of each race at high inoculum (5×10^8) was infiltrated into leaves of 271-4. For controls, leaves of ECW were inoculated with the same strains and leaves of ECW-10R were infiltrated with a strain of race 2.

Each of three fully expanded leaves below the first fork of six or seven plants of each line was infiltrated with inocula in nonoverlapped areas of 4–5 cm^2. After inoculation, the plants were kept in a growth chamber at 30 C in one experiment and 25 C in another. At predetermined times after inoculation, six disks of tissue (0.5 cm^2 each) were removed from an inoculated area, suspended in 3 ml of deionized water, and infiltrated with water by application and release of a vacuum (63 cm Hg for 1 min). Conductivity (μmhos) of the suspending solution was recorded immediately after submersion of disks and again after infiltration and agitation for 1 hr at 30 C. The difference in conductivity between the two readings represented the interaction of bacteria with host tissue.

RESULTS

All 16 strains of the pepper group of *X. c.* pv. *vesicatoria* collected in Florida caused typical susceptible reactions in plants of ECW. Necrosis occurred 2–3 days after infiltration of the suspensions of 5×10^8 cfu/ml into the leaves. Eight of the strains caused an HR in plants of ECW-10R and were classed as strains of race 2. The other eight strains were presumed to be race 1, based on development of a susceptible reaction in leaves of ECW-10R. The race 2 strains, XV 61-38, XV 65-1, XV 70-7, XV 80-6, XV 81-23, XV 82-7, XV 83-3, and XV E-3, induced an HR in 65 of 166 plants of PI 271322. Of the presumptive race 1 strains, XV 0623, XV 71-21, XV 80-5, and XV 82-8 caused an HR in 123 of the 166 plants, but four of the strains, XV 69-1, XV 77-3A, XV 81-18, and XV 82-15, did not produce a typical HR in any plant. These strains were different from any that had been described previously and were designated strains of race 3.

Although a typical HR did not occur in any of the plants of PI 271322 inoculated with the strains of race 3, a typical susceptible reaction also did not occur. A necrosis developed within 2–3 days after inoculation of leaves with suspensions of 5×10^8 cfu/ml, as with susceptible reactions, but the necrosis was not preceded by a water-soaked appearance and was dry and dark brown. Strains of all races caused these symptoms in plants of PI 271322 if a typical HR did not occur. These symptoms were typical of the quantitatively assessed resistance that appears to be homogenous in plants of PI 271322.

Plants were selected that were resistant to all three races of the pathogen. One of these plants, selected by progeny testing, was homozygous for all three resistances and was designated 271-4. Progeny of this plant were used in subsequent experiments.

Bacterial populations. In leaves of ECW inoculated with 5×10^3 cfu/ml, populations of all three races increased to 10^7–10^8 cfu/cm^2 of leaf area by 14 days. In leaves of 271-4, the population of the strain of race 2 increased for 2 days, or until cell numbers reached 5×10^3 cfu/cm^2, and then the population declined. The strain of race 1 also increased for 2 days, but the population reached 1×10^4 cfu/cm^2 before declining. The number of cells of race 3 increased for 6 days in 271-4, or until the population reached 1×10^5 cfu/cm^2, and then declined (Fig. 1).

No large or consistent differences in bacterial multiplication were observed in the reciprocal hybrids, and data from them were pooled. In the hybrids, the population dynamics of the strain of race 1 were closer to those of 271-4 than of ECW. On the other hand, the population dynamics of the strain of race 2 were intermediate between the two parents, and that of the strain of race 3 was closer to the susceptible parent. Apparently, various degrees of dominance occur with the resistance genes.

Electrolyte leakage. Consistently greater conductivity occurred at each sampling time with plants held at 30 C than with plants held at 25 C. No interaction of races and resistance genes occurred with the two temperatures; therefore, the means for both temperatures were averaged (Fig. 2). Conductivity of solutions containing the inoculated disks from ECW leaves was progressively greater in samples collected over a 48-hr period. Increases in conductivity with time occurred with all three races and were gradual, as expected for a susceptible reaction. Maximum electrolyte leakage occurred in the least amount of time after inoculation of leaves of ECW-10R with a strain of race 2, and a similar pattern was obtained with the same strain infiltrated into leaves of 271-4. The time to maximum leakage of electrolytes in leaves of 271-4 inoculated with the strain of race 1 was about 12 hr longer than that for the race 2 strain in the same plant and 24 hr before maximum leakage of electrolytes from susceptible leaves, i.e., ECW

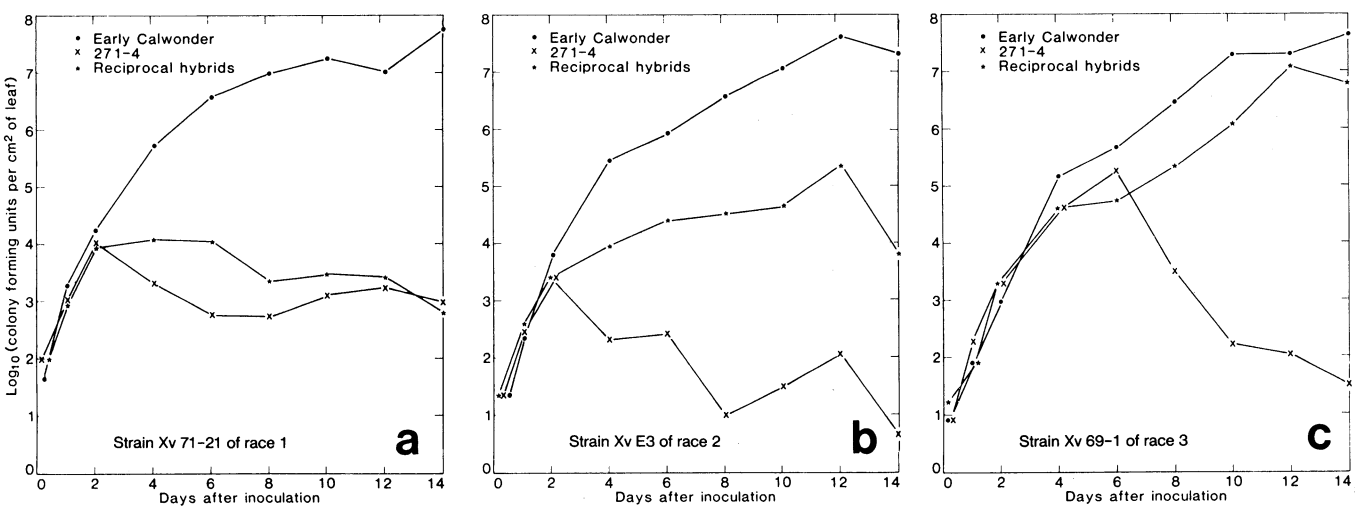

Fig. 1. Populations of bacteria per square centimeter of leaf tissue at increasing times after inoculation. Early Calwonder, 271-4, and their reciprocal hybrids were inoculated with a strain of **(A)** race 1, **(B)** race 2, and **(C)** race 3, each at 5×10^3 cells per milliliter.

leaves inoculated with the same strain. The pattern of electrolyte leakage from leaves of 271-4 inoculated with the strain of race 3 was similar to that of susceptible leaves inoculated with the same strain.

No large or consistent differences in electrolyte leakage occurred between reciprocal hybrids inoculated with a particular strain, and the data for each strain were pooled. The time interval from inoculation to maximum leakage for the hybrids inoculated with a strain of race 2 was between that for the parents but resembled that of the resistant parent more closely than the susceptible one (Fig. 3). Electrolyte leakage in the hybrids inoculated with race 1 was similar to that for the resistant parent.

DISCUSSION

The HR type of resistance in pepper plants to *X. c.* pv. *vesicatoria* probably results from a gene-for-gene interaction (7). Three single genes for resistance to *X. c.* pv. *vesicatoria* in pepper have been demonstrated in inheritance studies, and the presence of one avirulence gene in the bacterium has been demonstrated to be on a self-transmissible plasmid (13). In the future, it will be better to define races of the pathogen as those strains that have particular avirulence genes. However, such a definition of races should wait until the avirulence genes have been demonstrated by genetic recombinant techniques. This is in progress with the *X. c.* pv. *vesicatoria*-pepper system.

It has been demonstrated in other work that loss of the avirulence gene that determines race 2 converts the strain to race 3 (R. E. Stall, *unpublished*). One could predict that the loss of a possible avirulence gene that determines race 1 would also convert the strain to race 3. However, this does not have to be the case because avirulence genes responsible for the race 1 and race 2 determinants could be in the same strain of *X. c.* pv. *vesicatoria*, and loss of only one of them would not convert the strain to race 3. However, strains that contain both avirulence genes have not yet been isolated from nature.

The third resistance (quantitatively assessed) in plants of PI 271322 is thought to be a hypersensitive resistance. Although the electrolyte leakage pattern from leaves with this resistance was similar to that from susceptible leaf tissue, the population dynamics of the pathogen in the resistant and susceptible leaf tissue were clearly different. The pathogen's population in the quantitatively assessed resistant leaves increased as in susceptible leaves, then abruptly stopped and declined, while the pathogen continued to increase in susceptible leaves. A similar pattern occurred with the typical HR involving the *Bs*1 and the *Bs*3 genes and incompatible races. However, in the typical HR, the pathogen stopped growth much sooner after invasion of the leaves. Importantly, the incompatible pathogen reached a higher population in leaves with the quantitatively assessed resistance than did the incompatible strains that induced the typical HR, but the population in the leaves with the quantitatively assessed resistance was still less than in susceptible leaves. It is our opinion that the bacterial population dynamics is a better indicator of HR than is the time to necrosis after inoculation.

The HR associated with the quantitatively assessed resistance was slow in development as demonstrated by electro-

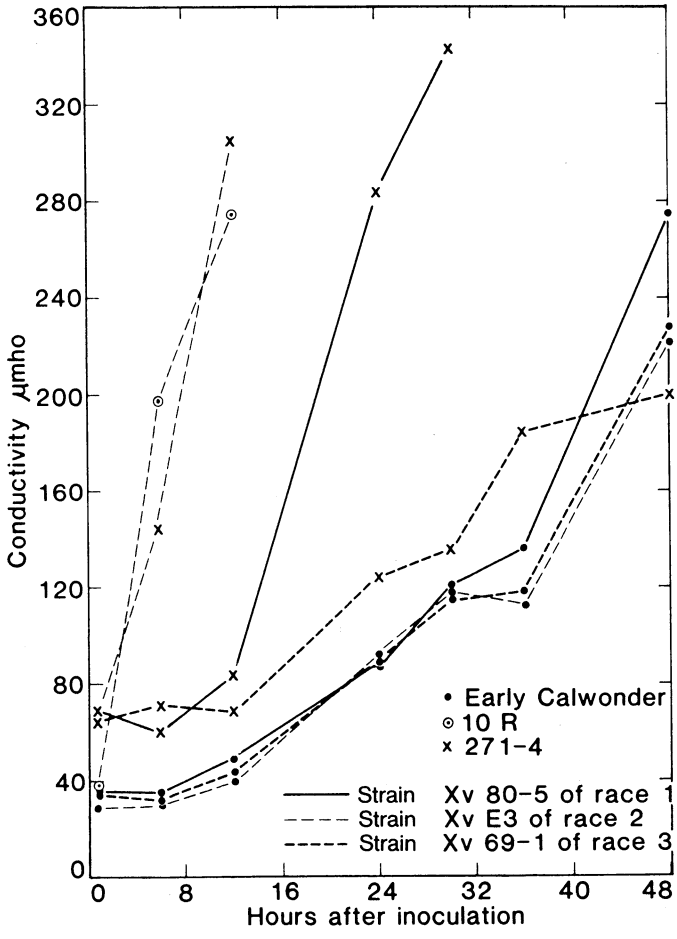

Fig. 2. Conductivity as a measure of electrolyte loss from tissue of pepper leaves inoculated with strains of races 1, 2, and 3 of *Xanthomonas campestris* pv. *vesicatoria* at concentrations of 5×10^8 cells per milliliter. Data were averaged for temperature treatments of 25 and 30 C.

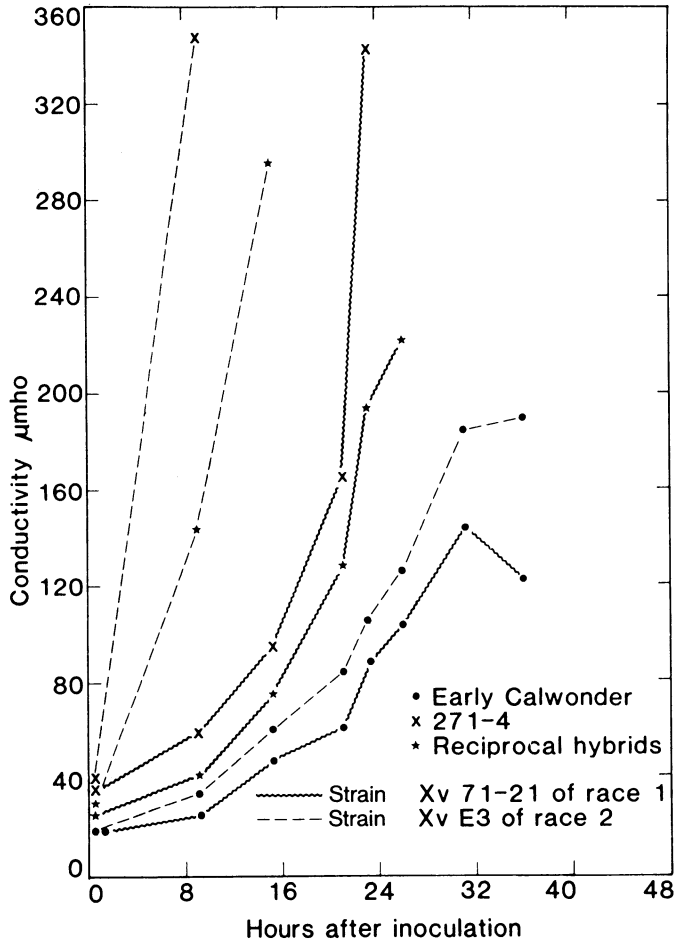

Fig. 3. Conductivity as a measure of electrolyte loss from tissue of pepper leaves inoculated with races 1 and 2 of *Xanthomonas campestris* pv. *vesicatoria* at concentrations of 5×10^8 cells per milliliter. Early Calwonder, 271-4, and their hybrids were inoculated.

lyte leakage. The slow development of the HR allowed the pathogen to increase to relatively high levels, and often, a few small lesions form after inoculation with low concentrations of bacteria. Selection of resistant plants can be accomplished from determinations of the number and size of lesions. Such quantitative variables are influenced by environmental factors, and a range of values often results, even with homozygously resistant plants. Therefore, the quantitatively assessed aspect of the resistance results in continuous data, which would appear to support a polygenic inheritance of the resistance. Because of this, the polygenic nature reported for the quantitatively assessed resistance in PI 271322 should be reevaluated (11).

The knowledge that different combinations of resistance genes and incompatible races can have different phenotypes is not new. This has been reported in cowpea inoculated with *X. campestris* pv. *vignicola* (8). However, the full range of phenotypic variation may not be realized, and the significance of the different phenotypes may not be completely understood. Some slow-forming HR resistances can be completely overlooked if high inocula are used in screening procedures. That is because necroses develop at about the same time as they do in susceptible plants. If the slow-forming HR resistances are detected by low inoculum doses, they may be discarded because they are assumed to be polygenic in inheritance and too complicated for inclusion in breeding programs. We believe other slow-forming HR resistances exist in the pepper gene pool and may be important in development of resistant cultivars that have durable resistance.

LITERATURE CITED

1. Cook, A. A., and Guevara, Y. G. 1982. Characterization of pepper strain race 1 of *Xanthomonas campestris* pv. *vesicatoria*. (Abstr.) Phytopathology 72:356.
2. Cook, A. A., and Guevara, Y. G. 1984. Hypersensitivity in *Capsicum chacoense* to race 1 of the bacterial spot pathogen of pepper. Plant Dis. 68:329-330.
3. Cook, A. A., and Stall, R. E. 1963. Inheritance of resistance in pepper to bacterial spot. Phytopathology 53:1060-1062.
4. Cook, A. A., and Stall, R. E. 1968. Effect of *Xanthomonas vesicatoria* on loss of electrolytes from leaves of *Capsicum annuum*. Phytopathology 58:617-619.
5. Cook, A. A., and Stall, R. E. 1969. Differentiation of pathotypes among isolates of *Xanthomonas vesicatoria*. Plant Dis. Rep. 53:617-619.
6. Cook, A. A., and Stall, R. E. 1982. Distribution of races of *Xanthomonas vesicatoria* pathogenic on pepper. Plant Dis. 66:388-389.
7. Flor, H. H. 1955. Host-parasite interactions in flax rust—its genetics and other implications. Phytopathology 45:680-685.
8. Gitaitis, R. D. 1983. Two resistant responses in cowpea induced by different strains of *Xanthomonas campestris* pv. *vignicola*. Plant Dis. 67:1025-1028.
9. Hibberd, A. M., Bassett, M. J., and Stall, R. E. 1987. Allelism tests of three dominant genes for hypersensitive resistance to bacterial spot of pepper. Phytopathology 77:1304-1307.
10. Kim, B. S., and Hartmann, R. W. 1985. Inheritance of a gene (Bs_3) conferring hypersensitive resistance to *Xanthomonas campestris* pv. *vesicatoria* in pepper (*Capsicum annuum*). Plant Dis. 69:233-235.
11. Stall, R. E. 1981. Selection for a component of horizontal resistance to bacterial spot of pepper. Pages 511-517 in: Proc. Int. Conf. Plant Pathog. Bact. 5th. J. C. Lozano, ed. CIAT, Cali, Colombia. 640 pp.
12. Stall, R. E., and Cook, A. A. 1966. Multiplication of *Xanthomonas vesicatoria* and lesion development in resistant and susceptible pepper leaves. Phytopathology 56:1152-1154.
13. Stall, R. E., Loschke, D. C., and Jones, J. B. 1986. Linkage of copper resistance and avirulence loci on a self-transmissible plasmid in *Xanthomonas campestris* pv. *vesicatoria*. Phytopathology 76:240-243.