Bacterial spot on pepper caused by *Xanthomonas campestris* pv. *vesicatoria*, Race 2, from the south coast of Puerto Rico[1,2]

Mildred Zapata[3]

ABSTRACT

The incidence and symptomatology of the bacterial spot on pepper (*Capsicum annuum* L.) and the identification of the pathogenic race of *Xanthomonas campestris* pv. *vesicatoria* (Xcv) (Doidge) Dye involved in the disease complex were studied in commercial and experimental plantations on the south coast of Puerto Rico. Evaluations were made in Juana Díaz, Salinas and Santa Isabel. In commercial plantations cultivar Key Largo showed greater susceptibility than bell pepper. In the experimental plantations, pepper introductions varied in their reaction to Xcv from resistant to susceptible. Xcv isolated from pepper leaves and fruit tissue produced the following symptoms: necrotic lesions with chlorotic halos, raised hyperplastic pustule lesions (open and broken), raised canker-like lesions with chlorotic margins, raised canker lesions with necrotic borders and progressive water soaking. Bacterial strains collected from disease foci in commercial pepper fields were inoculated on the differential lines derived from the susceptible Early Cal Wonder (ECW). On the basis of reactions in the differentials ECW-10R, ECW-20R, and ECW-30R carrying the Bs_1, Bs_2 and Bs_3 resistance genes, respectively, all the Xcv pepper strains found on the southern coast of Puerto Rico belong to race 2. The absence of other pathogenic races indicates that Xcv race 2 produces diverse symptomatology in peppers in Puerto Rico.

Key words: bacterial spot races, pepper, *Xanthomonas campestris* pv. *vesicatoria*, bacterial spot symptoms

RESUMEN

Mancha bacteriana del pimiento causada por *Xanthomonas campestris* pv. *vesicatoria*, raza 2, en la costa sur de Puerto Rico.

Se estudió la incidencia y sintomatología de la mancha bacteriana del pimiento, *Capsicum annuum*, y se identificó la raza 2 de *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) en plantaciones afectadas a nivel comercial y experimental en Juana Díaz, Salinas y Santa Isabel, Puerto Rico. En las siembras comerciales el híbrido Key Largo mostró mayor susceptibilidad que el pimiento morrón bajo iguales condiciones de siembra en las tres localidades. En las siembras experimentales las introducciones de pimiento mostraron reacciones que variaron desde resistentes a susceptibles. *Xcv*

[1]Manuscript submitted to Editorial Board 17 March 1993.

[2]This research was supported by special project SP-248 (Identification of resistance in pepper lines to bacterial spot in Puerto Rico) and HATCH-369 (Bacterial diseases of edible crops and ornamental plants in Puerto Rico). The author thanks Mr. Melvin Irizarry and Dr. Fernando Gallardo for providing information about location of the commercial pepper plantations and non-pesticide treated plantations, respectively.

[3]Associate Bacteriologist, Department of Crop Protection.

se aisló de tejidos cuyos síntomas variaron desde lesiones necróticas con halos cloróticos, pústulas elevadas (abiertas o expuestas), cancros elevados con márgenes cloróticos, cancros elevados con bordes necróticos y edema progresivo. Los aislamientos de Xcv coleccionados de los diferentes focos de infección se inocularon en plantas diferenciales derivadas de cruces con otras fuentes de resistencia y la línea susceptible Early Cal Wonder (ECW). Las reacciones a *Xcv* en los diferenciales ECW-10R, ECW-20R y ECW-30R portadores de los genes de resistencia Bs_1, Bs_2 y Bs_3, respectivamente, mostraron que todos los aislamientos de *Xcv* estudiados originados de tejidos de pimiento con síntomas diferentes en la costa sur de Puerto Rico pertenecen a la raza 2 de *Xcv*.

INTRODUCTION

Bacterial spot of pepper caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge, 1920) Dye 1978 (*Xcv*) is a common and destructive disease of peppers, (*Capsicum annuum* L.). Other natural hosts are *Capsicum* spp., *C. frutenscens* L. (chili peppers), *Lycopersicon esculentum* Mill. (tomato) and *Solanum tuberosum* L. (potato) (Ercolani, 1972; Kuprevicz, 1951). *Solanum melongena* L. can be a host by artificial inoculation.

The bacterium is widespread in North and South America, Africa, Australia and Europe (Hayward and Waterston, 1964) and infects leaves, stems, fruits and seeds. It causes irregular greasy spots which become dark tan, frequently with yellow margins, on leaves and stems. The leaves often turn yellow and fall. Fruit spots are circular, tan, slightly sunken with a central scab (Mingtan and Watson, 1973).

In Puerto Rico, preferred cultivars are susceptible to *Xcv* (Ruiz et al., 1992). Neither the use of certified seed nor of bactericides has been effective in controlling bacterial spot. Successful disease control may depend on the identification of pathogenic race(s) and use of specific disease resistance genes in a breeding program. This study was directed at determining the incidence and symptomatology of the bacterial spot and to the identification of the metabolic characterization and pathogenic variability of *Xcv* in peppers in Puerto Rico.

MATERIALS AND METHODS

Five commercial pepper fields planted with cultivar Key Largo and separated from each other by at least one kilometer were evaluated for bacterial spot in Juana Díaz, Salinas, and Santa Isabel. Cupric hydroxide (Kocide 101, 3.6 g/L) was sprayed weekly to control *Xcv*. Leaves and fruits showing the bacterial spot symptoms were collected from 10 infection foci within each field. Several diseased tissues were observed under laboratory conditions. Bacterial isolations were made on yeast dextrose calcium carbonate agar (YDCA) and the isolates were further inoculated on differential plants under greenhouse conditions as described later. Several standard determinative tests for the organism were used to characterize the bacteria, including cell morphology, Gram reaction, KOH, flagellation, catalase, oxidase, nitrate reductase, acid production from sugars, utilization of asparagine as a sole source of carbon and nitrogen and xanthomonadin pigment detection (Shaad and Stall, 1988).

To produce metabolic fingerprints, strains were grown on nutrient agar, suspended in sterile saline and adjusted to 0.38 at A = 590 and suspensions were inoculated into Biolog GN Micro Plates (150 µl per well) and incubated at 28° C. Two replicates were done per strain. Each plate was read at 24, 48 and 72 h. Metabolic profiles of other xanthomonads, such as *X. campestris* pv. *phaseoli* and *Xc.* pv. *dieffenbachiae*, were obtained for comparison with *Xcv* by the same procedure. Reactions in the wells were evaluated with the Microlog software. The software evaluates the quality of each identification as follows: $0.000 < Sim < 0.500$, no identification; $0.501 \leq Sim \leq 1.000$, good identification.

Two experimental field plots consisting of a germplasm collection of *Capsicum* spp. were evaluated in Juana Díaz. No pesticides (bactericide, fungicide or insecticide) were applied to the plot. A completely randomized design with four replicates of five plants each was used. Disease symptoms were recorded throughout the growing season. Disease reactions were determined on leaves of 2- to 4-month old plants by using a scale of 0-5, where 1 = no infection, 2 = slightly susceptible, 3 = moderately susceptible, 4 = susceptible and 5 = highly susceptible. Diseased tissue was collected from each germplasm line that showed symptoms. Isolations were made on YDCA and then inoculated on the differential lines under greenhouse conditions.

The susceptible pepper cultivar Early Calwonder (ECW) and three pepper lines, Early Calwonder-10R (ECW-10R), Early Calwonder-20R (ECW-20R) and Early Calwonder-30R (ECW-30R) carrying respectively, the Bs_1, Bs_2 and Bs_3 resistance genes (Hibberd et al., 1987), were used under greenhouse conditions to differentiate *Xcv* races within the pepper isolates in Puerto Rico. Differential lines and pathogenic *Xcv* strains of the pepper group, race 1, and the pepper-tomato group, races 2 and 3, were obtained from Drs. J. B. Jones and R. E. Stall (University of Florida at Homestead). Bacterial cultures were grown for 24 h on YDCA. Twelve bacterial isolates from the commercial fields were selected for race identification. Inoculation of the differential lines was done with sterilized needles (1 mm diameter) impregnated with bacteria under greenhouse conditions at La Finca Alzamora, Mayagüez, Puerto Rico. A completely randomized design with two replicates was used for the greenhouse experiment. Each plant was inoculated three times with each bacterial strain.

The adaxial surface of each leaf was inoculated with a sterile needle impregnated with bacteria at three sites on one side of the midrib. The other half of the midrib was inoculated with the sterile needle (1 mm diameter) as a control. Lesion progression was recorded in millimeters.

RESULTS AND DISCUSSION

The bacteria showed typical characteristics of xanthomonads. They were yellow, rod-shaped, aerobic, gram negative, KOH positive, oxidase negative, catalase positive, and then used glucose, arabinose, mannose, trehalose, and cellobiose for acid production. None used asparagine, and were polarly flagellated. Xanthomonadin pigment showed an absorption maxima between 442 and 449 mm.

Metabolic fingerprints

The Biolog metabolic fingerprinting technique was useful for the identification of *Xcv* pepper strains. The metabolic fingerprints of *X. campestris* pv. *vesicatoria* (*Xcv*) strains, associated with different symptoms in peppers were reproducible. The similarity index using data from 48- and 72-h incubation of GN plates varied from 0.657 to 0.898.

Carbon sources utilized by all the *Xcv* strains tested were D-melibiose, bromosuccinic acid, D-fructose, cis-aconitic acid, succinamic acid, dextrin, L-fucose, citric acid, glycogen, D-galactose, D-raffinose, alaninamide, Tween 40, gentiobiose, D-alanine, Tween 80, α-D-glucose, L-alanine, L-proline, sucrose, L-pyroglutamic acid, N-acetyl-D-glucosamine, D-trehalose, lactulose, glycerol, maltose, L-glutamic acid, methyl pyruvate, cellobiose, mono-methyl succinate, succinic acid, and glycyl-L-glutamic acid.

Carbon sources utilized by none of the *Xcv* strains tested were α-cyclodextrin, N-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol-i-erythritol, m-inositol, α-lactose, D-mannitol, B-methyl glucoside, psicose, L-rhamnose, D-sorbitol, turanose, xylitol, formic acid, D-galactonic acid, β-hydroxybutyric acid, itaconic acid, α-keto butyric acid, α-keto valeric acid, quinic acid, D-saccharic acid, sebacic acid, glucuronamide, L-asparagine, L-aspartic acid, L-histidine, L-leucine, L-ornithine, D-serine, D,L-carnitine, β-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, D,L-α-glycerol phosphate, glucose-1-phosphate, and glucose-6-phosphate.

Variable reactions were observed with acetic acid, hydroxy L-proline, α-keto butyric acid, α-keto glutaric acid, α-keto valeric acid, Lphenyl alanine, D, L-lactic acid, malonic acid, propionic acid, L-serine, L-hydroxy butyric acid, L-threonine, B-hydroxy butyric acid, D-mannose.

Field evaluation

All Key Largo plantations were highly susceptible to *Xcv.* Bell peppers were moderately susceptible in comparison with Key Largo. Bacterial pustules were more common in bell pepper than in Key Largo. An infection grade of 4-5 was common in all Key Largo fields where copper compounds were used to control *Xcv.* Disease severity was lower in those plots in which preventive chemical treatments were applied.

In the *Capsicum* spp. introductions, disease reactions ranged from susceptible to resistant.

Symptomatology

The most frequently found symptoms were characterized by necrotic lesions with chlorotic halos (Figure 1a). *Xcv* could also be isolated from raised and closed hyperplastic pustules (Figure 1b), from raised cankerlike lesions (Figure 1c) and from raised and broken hyperplastic pustule lesions (Figure 1d). The formation of necrotic lesions with chlorotic margins seems to be more detrimental to the physiological stage of the plant than the pustules. Defoliation was more common on leaves with necrotic lesions than on those with pustules. Pustule reaction seems to spread slowly and develop in high frequency without inducing defoliation. The formation of cankers and subsequent rupture of epidermal layers leading to bacterial oozing were not associated with defoliation (Figure 1e). However, this symptom may be more important for the dispersal of the bacterium from the leaf surface than the pustules. Pustules maintain their integrity without releasing bacteria. This characteristic may represent a better survival mechanism for the pathogen.

Race determination

Xanthomonas campestris pv. *vesicatoria* is widely distributed throughout the world. Race 1 is frequent in pepper production areas around the world. Race 2 (the pepper strain) has been found in Guadeloupe, Florida and Brazil (Cook and Stall, 1982) and speculated to have originated as a mutant form of Race 1. Within the pepper-tomato group, Race 3 has been found in Florida and infects pepper ECW, ECW-10R, ECW-30R and tomato (*Lycopersicon esculentum* L). Among the

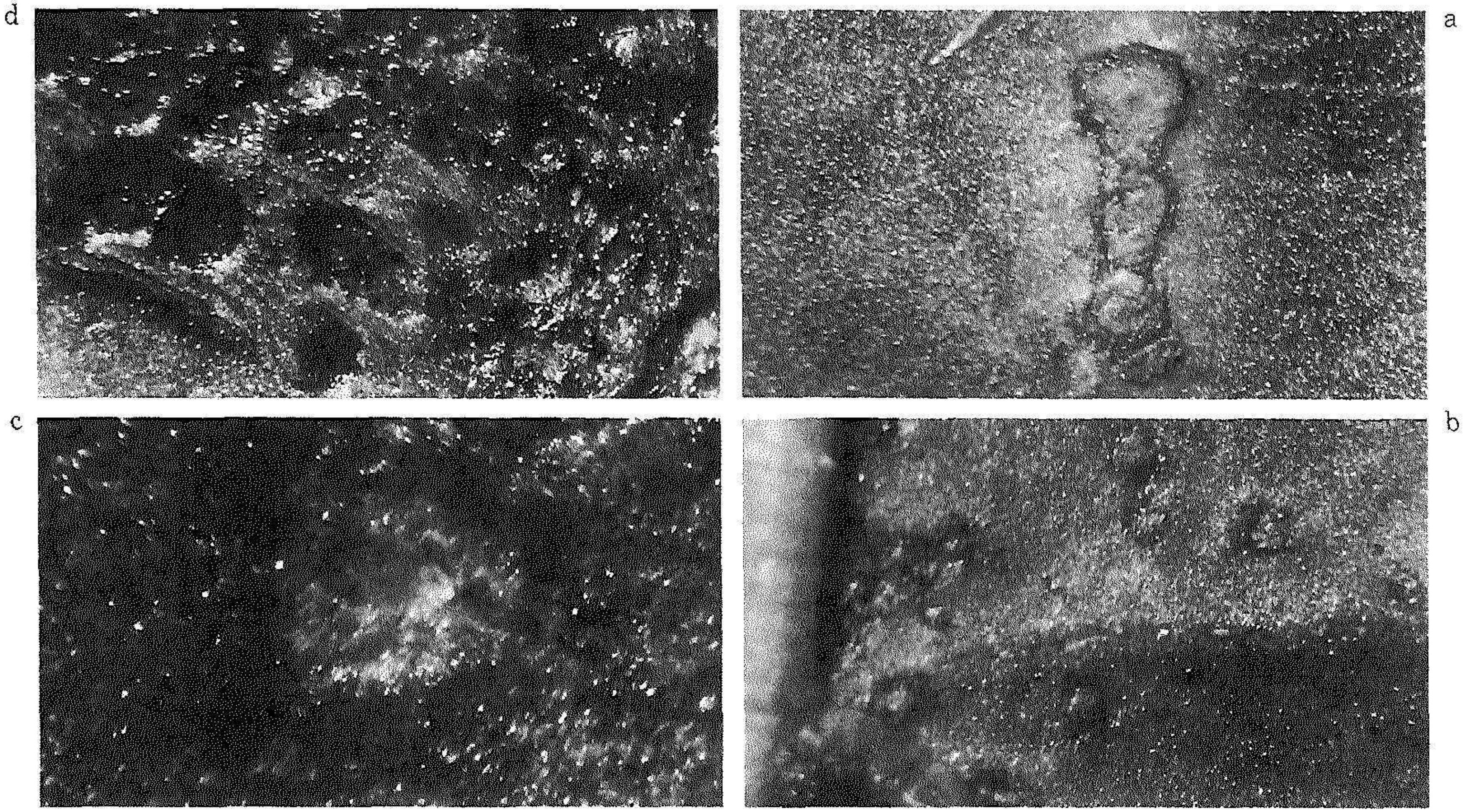

FIGURE 1. Bacterial spot leaf symptoms on field grown peppers; clockwise from top right: a): Necrotic lesion with chlorotic halos; b) raised and closed hyperplastic pustule lesions; c) raised canker like lesion with chlorotic margins; d) raised and broken hyperplastic pustule lesion.

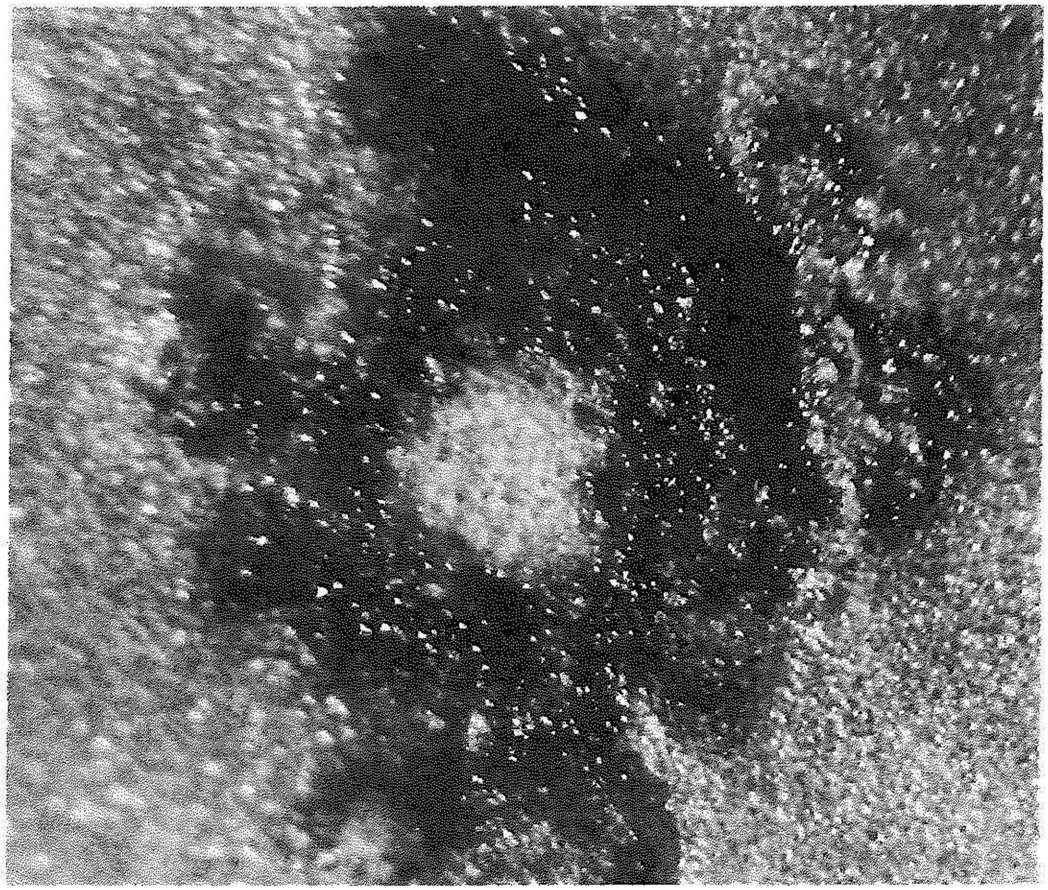

FIGURE 1e. Raised canker lesion with necrotic borders and progressive watersoaking.

differential lines, the most virulent race was Race 3, followed by Race 1 (Table 1). Race 2 was avirulent on ECW-10R and ECW-20R but was virulent on ECW-30R.

In Puerto Rico *Xcv* was reported in tomato in 1961 (Pérez and Cortés-Monllor, 1961); however, race was not identified. Twelve *Xcp* pepper strains from 1991 commercial plantings on the south coast of Puerto Rico were avirulent on ECW-10R and ECW-20R, which contain resistance genes Bs_1 and Bs_2, respectively, and slightly virulent on ECW and ECW-30R (Table 2). The results support the identity of the *Xcv* pepper strains collected from five commercial and experimental areas in Puerto Rico as race 2 (Figure 2). In contrast, Dr. J. Jones of the University of Florida, Homestead, analyzed a collection which comprises strains from the Caribbean and other countries and did not find Race 2 in Puerto Rico (personal communication).

In southern Florida during the 1989-90 and 1990-91 seasons, race 1 was the most frequently found on pepper, followed by races 2 and 3 (Phoronezny et al., 1992). The majority of the strains found are copper

TABLE 1.—*Bacterial spot lesions caused by* Xanthomonas campestris *pv.* vesicatoria Xcv *(pathogenic races 1, 2 and 3) 36 days after inoculation on pepper leaves.*[1]

Xcv	Mean lesion size in mm on pepper lines[1]		
	ECW-10R	ECW-20R	ECW-30R
Race	Bs_1	Bs_2	Bs_3
1	14.66b[2]	1.00	1.00b
2	1.00c	1.00	4.66c
3	23.00a	1.00	23.33a

[1]Pepper ECW = Early Cal Wonder cultivar, ECW-10R refers to a line derived from ECW containing the resistance gene, Bs_1. Resistance genes are written below the respective pepper line's name, ECW-20R and ECW-30R.

[2]Mean of three replicates with numbers within columns followed by the same letter are not different according to the student 't' test (P = 0.05).

resistant. In contrast, only Race 2 was detected in the commercial pepper areas in southern Puerto Rico. Race 2 is not able to overcome Bs_1 and Bs_2 resistance genes, but it can overcome Bs_3 producing small lesions in cultivars carrying the gene. Commercial cultivars used in Puerto Rico seem to lack Bs_1 and Bs_2 resistance genes as indicated by their susceptibility to *Xcv*. A breeding program to improve resistance of pepper to *Xcv* should consider ECW-20R as a good source of genetic resistance. On the other hand, cultivars should be recommended to farmers on the basis of disease resistance to *Xcv* Race 2, which is the

TABLE 2.—*Race identification of the pepper group of* Xanthomonas campestris *pv.* vesicatoria *(Xcv) found on the south coast of Puerto Rico.*

Xcv		Virulence reaction on Pepper			
Origin	Race	ECW[2]	ECW-10R[3]	ECW-20R	ECW-30R
			Bs_1	Bs_2	Bs_3
P.R.[1]	2	+[4]	-	-	+
Florida	1	+	+	-	-
Florida	2	+	-	-	+
Florida	3	+	+	-	+

[1]P.R. = The field evaluation of 12 isolates of Xcv from the commercial plantations; Florida refers to the standard isolate (positive control) of each race.

[2]ECW = Early Calwonder cultivar.

[3]ECW-10R = A line derived from ECW containing the resistance gene, Bs_1. Resistance genes are written below the respective pepper line's name, ECW-20R and ECW-30R.

[4]+ = virulent strain; - = avirulent strain.

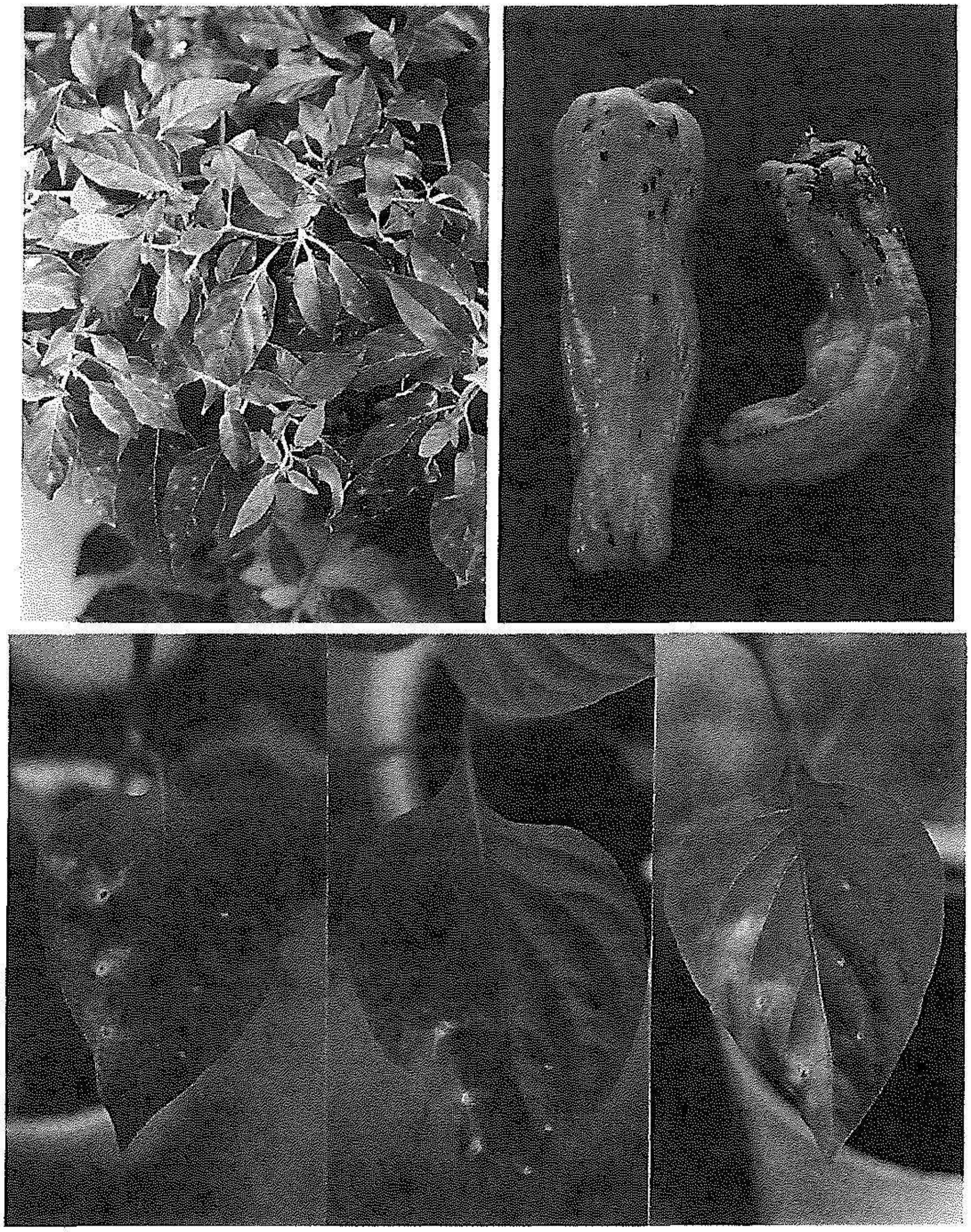

FIGURE 2. Top: Natural infection of bacterial spot disease on Key Largo pepper plants (left) and fruits (right) under field conditions. Bottom: Artificial inoculation showing at the left three inoculated and at right three control sites per leaf 36 days after inoculation. Left to right: ECW-10R reaction to Race 1; ECW reaction to Race 2 represented by a Puerto Rican isolate; and ECW-30R reaction to Race 3.

pathogenic race prevalent in Puerto Rico. Chemical control, its related costs, and the amount of released chemicals into the environment will be minimized if genes resistant to *Xcv* Race 2 are introduced. Also since the use of copper based bactericides in Florida and other countries has led to copper resistance, chemical control may confront an efficacy problem in the near future in Puerto Rico. Improved varieties with *Xcv* resistance would provide a more environmentally compatible and cost effective strategy for bacterial spot control.

LITERATURE CITED

1. Cook, A. A. and R. E. Stall, 1982. Distribution of races of *Xanthomonas vesicatoria* pathogenic on pepper. *Plant Disease* 66:388-89.
2. Ercolani, G. L., 1972. A bacterial spot of potato caused by *Xanthomonas vesicatoria* in Italy. *Review of Plant Pathology* 51:611 (Abstract).
3. Hayward, A. C. and J. M. Waterston, 1964. *Xanthomonas vesicatoria*. C.M.I. Descriptions of pathogenic fungi and bacteria. No. 20.
4. Hibberd, A. M., M. J. Bassett and R. E. Stall, 1987. Allelism tests of three dominant genes for hypersensitive resistance to bacterial spot of pepper. *Phytopathology* 77:1304-07.
5. Kuprevicz, V. F., 1951. The physiology of the diseased plant in relation to the general questions of parasitism. *Review of Plant Pathology* Vol. 30:61 (Abstract).
6. Mingtan, L. and T. Watson, 1973. Bacterial spot of tomato and pepper in California. *Plant Disease Reporter* 57:258-259.
7. Pérez, J. E. and A. Cortés-Monllor, 1961. *Xanthomonas vesicatoria* in Puerto Rico. *J. Agric. Univ. P.R.* 45:304-10.
8. Pohronezny, K., R. E. Stall, B. I. Canteros, M. Kegley, L. E. Dantoff and R. Subramanya, 1992. Sudden shift in the prevalent race of *Xanthomonas campestris* pv. *vesicatoria* in pepper fields in the southern Florida. *Plant Dis.* 76:118-20.
9. Ruiz, H., G. Fornaris and E. Caraballo, 1992. Resistencia de seis cultivares comerciales de pimiento de cocinar (*Capsicum annuum* L.) a la mancha bacteriana causada por *Xanthomonas campestris* pv. *vesicatoria* (Doigde) Dye *J. Agric. Univ. P.R.* 76:97-100. Res. Note.
10. Schaad, N. W. and R. E. Stall, 1988. *Xanthomonas*. Pages 81-94 In: Laboratory Guide for Identification of Plant Pathogenic Bacteria. 2nd ed. N.W. Schaad, ed. American Phytopathological Society, St. Paul, MN.