Research Note

GROWTH REGULATOR MIX REDUCES LEAF LESIONS ON PEPPER (CAPSICUM ANNUUM L.) CAUSED BY XANTHOMONAS CAMPESTRIS *PV.* VESICATORIA[1,2]

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J. Agric. Univ. P.R. 86(3-4):163-167 (2002)

Bacterial spot on pepper, caused by *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) (Doidge) Dye, is one of the most serious diseases of pepper plants on the south coast of Puerto Rico. This disease affects yield and quality of fresh market peppers. Bactericides have not been effective in the control of this disease. New physiological, biological or genetic approaches must be developed for the reduction of the disease.

Bacterial entry and infection, disease development, and the role of stomata during infection must be better understood. Natural openings for bacterial pathogens include stomata, hydathodes, lenticels and nectarthodes (Diachum et al., 1942; Meier, 1934; Mew et al., 1984; Nelson and Dickey, 1970; Rich, 1983) and are considered relevant in the etiology of bacterial diseases because the pathogen cannot breach intact leaf surfaces (Wood, 1967). *Xcv* can enter leaves through mechanically produced epidermal wounds (Vakili, 1967). *Xcv* are residents of the leaf surface and probably can be splashed to other leaves before infection occurs through stomata (Leben, 1963). A possible relationship between stomata opening, density of stomata and resistance of stomata to pathogen entry has been suggested. However, this possibility has been documented in only a few instances (Rich, 1983; Welmer, 1983; and Wood, 1967). Hormones and growth regulators can affect stomatal operation, and thus may influence bacterial invasion.

This paper describes the effect of growth regulator treatments, applied to foliage and soil, on the severity of bacterial leaf spot on foliage of three pepper cultivars: Cayman, Biscayne and Key Largo.

A two factor randomized complete block design consisting of five treatments, four replicates, three pepper cultivars (Cayman, Biscayne and Key Largo) with 40 plants per treatment per replicate was used. A factorial ANOVA for the factors cultivar and treatment was done using the program MSTAT. The experiment was in the field from the months of March to June 1992 at Juana Díaz, Puerto Rico. Planting distance was 0.3 m within the row and 0.9 m between rows. Water was supplied by drip irrigation.

Growth regulator treatments were applied early in the morning (between 7:00 and 8:00 am) at the minimal dosage recommended for vegetables. The dosage, number of applications and time of application for each treatment were: 1) PGR IV[6] at 124.8 ml/ha, three applications, starting two weeks after transplanting, then at first flowering, and then at 21-day intervals; 2) Triggrr foliar at 566.4 ml/ha, six applications, starting at

[1]Manuscript resubmitted to Editorial Board 25 September 2002.

[2]This research was supported by HATCH-369 (Bacterial diseases of edible crops and ornamental plants in Puerto Rico).

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transplanting, then seven days after transplanting and then at 14-day intervals; 3) Triggrr to the soil at 2,128.8 ml/ha, two applications, starting at transplanting and then 30 days after transplanting; 4) Triggrr foliar + Keyplex 250 at 566.4 ml/ha + 4,543.2 ml/ha, respectively, six applications, at transplanting, then seven days after and then at 14-day intervals; and 5) Cupric hydroxide as the control treatment.

Active ingredients of PGR IV were indolebutyric acid and gibberellic acid; active ingredients of Triggrr were mixed cytokinins. Keyplex 250, consisting of essential micronutrients and alpha keto amino acids, was added to the foliar treatment of Triggrr. The lowest recommended rate of Kocide 101 (cupric hydroxide) was applied weekly on all plants and was used as the control. This compound is recommended for fungal and bacterial disease control in Puerto Rico, but at present it has no effect on *Xcv*.

Two replicated samples were taken for estimating the number of bacterial spot lesions on the adaxial and abaxial surfaces of 30 leaves representing 30 plants of each pepper cultivar. Natural infection with the pathogen occurred during the seedling stage, and bacterial spot symptoms were observed in the control plants of all replicates, thus indicating that dissemination of the pathogen and plant infection was evenly distributed by the time of flowering. Leaf samples were taken during the reproductive stage and evaluated under a stereomicroscope. Pathogen identity was confirmed by isolation, purification, and pathogenicity determination on indicator plants and reactions to 95 carbon sources (Biolog Inc., Hayward, CA).

Bacterial spot of pepper, *Capsicum annuum* L., was significantly reduced by treatment with PGR IV growth regulator, which consists of indolebutyric and gibberellic acids and micronutrients. The other treatments compared were foliage applications of Triggrr (commercial mixture of citokinins), Triggrr plus Keyplex (micronutrients and aminoacids) and soil application of Triggrr.

Bacterial spot lesions were less frequent on the adaxial (upper) surface of the leaf than on the abaxial (lower) surface for all the cultivars and treatments tested (Table 1). Water soaked lesions developed earlier on abaxial surfaces. Later, the lesions became more chlorotic or necrotic and were observed on the adaxial surface.

Plants treated with PGR IV showed a lower number of bacterial spot lesions on both sides of the leaves of Key Largo and Cayman, but not of Biscayne (Table 1). Lesion counts were averaged over all three cultivars to compare the treatments in all the tested populations. PGR IV treatment resulted in the lowest number of lesions on both surfaces of the leaf. The number of lesions on treatments containing Triggrr was not different from that on the control, neither on the adaxial (upper) leaf surface nor on the abaxial (lower) surface (Table 2).

Maximum temperatures were above 30° C with high relative humidity during the experiment (Table 3). These conditions were optimal for the disease development.

Hormones have been associated with virulence in some pathogenic bacteria which are able to produce them. Hormone and growth regulator treatments may modify virulence and should be further investigated as treatments for control of bacterial diseases. Closure of stomata may be important for practical disease control in the field. Ramos and Valin (1987) found that bacterial spot in tomato caused by *Xcv* was significantly reduced when the stomata were closed before inoculation with the bacterium. Treatment of tomato with the growth regulator abscisic acid at 4M concentration reduced bacterial spot incidence by 33%. In other crops such as beans, Zapata, (1992) and Zapata et al. (1992)

[6]Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

TABLE 1.—*Number of bacterial spot (*Xanthomonas campestris *pv.* vesicatoria*) lesions that developed on the two leaf surfaces of pepper cultivars in the field at Juana Díaz, Puerto Rico, after treatment with growth regulators.*

	Number of bacterial lesions on leaf[1]					
	Key Largo		Biscayne		Cayman	
Treatment[2]	Lower	Upper	Lower	Upper	Lower	Upper
PGR IV	13.4[3,4] efghij	4.4 hij	14.2 defghi	6.4 ghij	12.9 fghij	2.5 j
Triggrr (foliar)	27.2 abc	6.8 ghij	26.7 abc	8.4 ghij	14.9 defgh	5.3 ghij
Triggrr (soil)	21.4 bcdef	6.7 ghij	34.6 a	7.0 ghij	13.9 defghi	5.6 ghij
Triggrr + Keyplex (foliar)	24.6 abcde	6.2 ghij	23.6 abcdef	3.6 hij	30.5 ab	9.8 ghij
Control (Kocide 101)	21.9 bcdef	6.8 ghij	16.6 cdefg	6.3 ghij	25.2 abcd	3.4 ij
LSD ($P \leq 0.05$)	11.34					

[1]Comparison was made between the lower (abaxial) and upper (adaxial) leaf surfaces of each of the three pepper cultivars.

[2] Plant growth regulator commercial trade name.

[3]Means followed by the same letters are not significantly different at $P \leq 0.05$.

[4]Each number represents the mean of 30 observations per cultivar per treatment on each tissue surface.

found that a reduction in the severity of the disease caused by *Xanthomonas campestris* pv. *phaseoli* was related to overproduction of hormones by the bacterium.

Plant surface represents the first line of defense against pathogens. Some structural defenses are present in the plant even before the pathogen comes in contact with the plant. Such structures include wax and cuticle, structure of epidermal cell walls, the size, location and shapes of stomata, and lenticels that hinder the advance of the pathogen.

TABLE 2.—*Bacterial spot lesions developed on three pepper cultivars caused by* Xanthomonas campestris *pv.* vesicatoria *after treatment with growth regulators under field conditions at Juana Díaz, Puerto Rico.*[1]

	Leaf tissue	
Treatment[2]	Upper (adaxial)	Lower (abaxial)
PGR IV	4.4 d[3]	13.5 bc
Triggrr (foliar)	6.8 cd	22.9 a
Triggrr (soil)	6.4 cd	23.3 a
Triggrr (foliar) + Keyplex	6.5 cd	26.2 a
Control (Kocide 101)	5.5 cd	21.2 ab
LSD $P \leq 0.05$	8.018	

[1]Pepper cultivars evaluated were Key Largo, Cayman and Biscayne. The bacterial disease incidence occurred naturally.

[2]Plant growth regulator commercial trade name.

[3]Means followed by the same letters are not significantly different at $P \leq 0.05$.

TABLE 3.—*Temperature, relative humidity and rain recorded at Juana Díaz, Puerto Rico, during the experiment.*

Month	Temperature (° C)		Relative humidity (%)	Rain (cm)
	Min	Max		
March	19.5	30.0	76	1.19
April	21.0	31.0	87	3.35
May	22.0	30.5	96	17.14
June	23.0	31.5	80	1.57

Bacteria enter leaf surface through stomata. The number of stomata is determined by the plant species. The number does not vary with season or environmental conditions.

Bacterial spot caused by *Xcv* is mostly a foliar disease, although it can develop on fruits and other plant parts. In plant diseases in which the pathogen infects the leaves, transpiration is usually affected. Hormones and growth regulators can affect transpiration. This effect is the result of destruction of at least part of the protection afforded by the leaf cells, and dysfunction of stomata.

Certain plant hormones have been shown to reduce certain pathogen infections of plants, e.g., tomato by *Fusarium*, potato by *Phytophthora*, and persimmon fruit by gibberellic acid, through the increase of disease resistance (Pérez et al., 1995). In virus disease control, gibberellic acid sprays are used for the field control of sour cherry yellow virus disease.

Stomata are more frequently found on the lower side of leaves. Bacterial populations are more protected on this leaf side than on the upper side. Also, the higher humidity, maintained on the lower versus upper side of leaf favors dissemination and proliferation of bacteria. Higher concentration of ethylene due to bacterial proliferation and activity can stimulate leaf abscission and defoliation, characteristic symptoms of bacterial spot disease. Depending on the defoliation rate, yields can be adversely affected.

Cupric hydroxide was applied as a recommended cultural practice. At present, it does not control bacterial spot on pepper; however, it seems to protect against fungi. *Xanthomonas campestris* pv. *vesicatoria* has developed resistance to the cupric hydroxide compound. *Xcv* has resistance to copper and streptomycin in its genes, which in some strains are plasmid-encoded (Bender et al., 1990; Cantros et al., 1989, 1995).

The number of bacterial spot lesions on the adaxial and abaxial surface of the control leaves showed no difference among Triggrr treatments, thus indicating lack of negative or positive interactions between the cytoquinins and the bacteria. *X. campestris* pv. *vesicatoria* produces low amounts of ethylene. Increase in ethylene production has been related to higher bacterial populations, higher leaf abscission and greater disease severity. Application of IAA or amino-oxyacetic acid reduces the production of ethylene and, consequently, disease severity and leaf abscission. PGR IV, containing the plant regulators indolebutyric and gibberellic acids significantly reduced bacterial spot disease under field conditions in some pepper cultivars, thus suggesting a new approach to controlling the disease. A long-term positive effect could be a reduction in the primary inoculum for future plantings, especially if we consider that each lesion may have around 10^{4-6} bacteria able to survive. Bacterial spot disease induces high defoliation and erratic yields. A relationship between number of lesions and yield loss needs to be established to determine the economic impact of the PGR IV treatment. PGR IV is an interesting growth regulator because of the hormones it has and also because it increases the nutrient uptake of calcium, whose role in disease resistance is well substantiated.

A separate experiment established in Lajas, Puerto Rico, confirmed the effect of PGR IV in reducing bacterial spot symptoms. A completely randomized design consisting of six treatments, four replicates, two pepper cultivars and thirty plants per treatment was used. The treatments included were PGR IV, Kocide 101, Top Cop, two biological treatments and a control treated with water. A reducing effect on the number of lesions was observed in cultivars Key Largo and Elisa when treated with PGR IV (M. Z., unpublished).

LITERATURE CITED

Bender, C. L., D. K. Malvick, K. E. Conway, S. George and P. Pratt, 1990. Characterization of p*Xv* 10A, a copper resistance plasmid in *Xanthomonas campestris* pv. *vesicatoria*. *Appl. Environ. Microbiol.* 56:170-175.

Cantros, B. I., G. I. Minsavage, D. R. Pring and R. E. Stall, 1989. Plasmid-encoded copper resistance in *Xanthomonas campestris* pv. *vesicatoria*. pp. 351-356 *In:* Z. Klement (ed.). Proc. Int. Conf. Plant Pathogenic Bacteria, 7th. Akademiai Kiado, Budapest, Hungary.

Cantros, B. I., G. V. Minsavage, J. B. Jones and R. E. Stall, 1995. Diversity of plasmids in *Xanthomonas campestris* pv. *vesicatoria*. *Phytopathology* 85:1482-1486.

Diachum, S., W. D. Valleau and E. M. Johnson, 1942. Relation of moisture to invasion of tobacco leaves by *Bacterium tabacum* and *Bacterium angulatum*. *Phytopathology* 32:379-387.

Leben, C., 1963. Multiplication of *Xanthomonas campestris* pv. *vesicatoria* on tomato seedlings. *Phytopathology* 53:778-781.

Meier, D., 1934. A cytological study of the early infection stages of black rot of cabbage. Bull. Torrey Bot. Club 61:173-190.

Mew, T. W., I. C. Mew and J. S. Huang, 1984. Scanning electron microscopy of virulent and avirulent strains of *Xanthomonas campestris* pv. *oryzae* on rice leaves. *Phytopathology* 74:635-641.

Nelson, P. E. and E. A. Dickey, 1970. Histopathology of plants infected with vascular bacterial pathogens. *Annual Review Phytopathology* 8:259-280.

Pérez, A., R. Ben-Arie, A. Dinoor, A. Genizi and D. Prusky, 1995. Prevention of black spot Disease in persimmon fruit by gibberellic acid and iprodione treatments. *Phytopathology* 85:221-225.

Ramos, L. J. and R. B. Valin, 1987. Role of stomatal opening and frequency on infection of *Lycopersicon* spp. by *Xanthomonas campestris* pv. *vesicatoria*. *Phytopathology* 77:1311-1317.

Rich, S., 1983. The role of stomata in plant diseases. pp. 102-114. *In:* I. Zelitch (ed.). Stomata and Water Relations in Plants. Conn. Agricultural Experimental Bull. No. 664, New Haven. 166 pp.

Vakili, N. G., 1967. Importance of wound in bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) of tomatoes in the field. *Phytopathology* 57:1099-1103.

Welmer, C. M., 1983. Stomata. Longman, Inc., New York. 166 pp.

Wood, R. K. S., 1967. Physiological Plant Pathology. Blackwell Scientific Publishers, Oxford and Edinburgh. 127 pp.

Zapata, M., 1992. Role of bacterial hormone in plant-pathogen interactions. 16th Scientific Research Congress. Inter American University, Arecibo, PR.

Zapata, M., A. K. Vidaver and R. Dam, 1992. Hormone production in *Xanthomonas campestris* pv. *phaseoli*. BIC. 35:44-45.