HORTSCIENCE 35(5):882–884. 2000.

Control of Bacterial Spot on Tomato in the Greenhouse and Field with H-mutant Bacteriophages

J.E. Flaherty[1], J.B. Jones[2], B.K. Harbaugh[3], G.C. Somodi[1], and L.E. Jackson[4]

Gulf Coast Research and Education Center, University of Florida, 5007 60th Street East, Bradenton, FL 34203

Additional index works. Xanthomonas campestris pv. *vesicatoria, Lycopersicon esculentum*

Abstract. **A mixture of host-range mutant (h-mutant) bacteriophages specific for tomato race 1 (T1) and race 3 (T3) of the bacterial spot pathogen, *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye was evaluated for biological control of bacterial spot on 'Sunbeam' tomato (*Lycopersicon esculentum* Mill.) transplants and field-grown plants for two seasons (Fall 1997 and Fall 1998). Foliar applications of bacteriophages were compared with similar applications of water (control) and of copper/mancozeb bactericides, the commonly used chemical control strategy for tomato seedling and field production. In 1997, the incidence of bacterial spot on greenhouse-grown seedlings was reduced from 40.5% (control) to 5.5% or 0.9% for bactericide- or bacteriophage-treated plants, respectively. In 1998, the incidence of bacterial spot was 17.4% on control plants vs. 5.5% and 2.7% for bactericide- and bacteriophage-treated plants, respectively, although these differences were not statistically significant at $P \leq 0.05$. Applications of bacteriophages to field-grown tomatoes decreased disease severity as measured by the area under the disease progress curve (AUDPC) by 17.5% (1997) and 16.8% (1998) compared with untreated control plants. Preharvest plant vigor ratings, taken twice during each field season, were higher in the bacteriophage-treated plants than in either bactericide-treated plants or nontreated controls except for the early vigor rating in 1998. Use of bacteriophages increased total weight of extra-large fruit 14.9% (1997) and 24.2% (1998) relative to that of nontreated control plants, and 37.8% (1997) and 23.9% (1998) relative to that of plants treated with the chemical bactericides. Chemical names used: manganese, zinc, carboxyethylene bis dithiocarbamate (mancozeb).**

Xanthomonas campestris pv. *vesicatoria* (*Xcv.*) causes bacterial spot disease on tomato and pepper (*Capsicum annuum* L. var. *annuum*) plants. This disease can be economically devastating to growers, especially in warm and damp climates (Pohronezny and Volin, 1983). Attempts to control this pathogen with a variety of chemical, biological, and genetic strategies have had limited success (Jones and Jones, 1985; Jones and Scott, 1986; Scott and Jones, 1986). Race shifts among populations of *Xanthomonas* sp. have thwarted genetic approaches for providing stable resistance in commercial cultivars of tomatoes and peppers (Jones et al., 1995; Kousik and Ritchie, 1996; Pohronezny et al., 1992). Copper-containing bactericides have been used to control bacterial spot for many years, but their effectiveness has been hampered by the emergence of copper-resistant strains of the pathogen (Jones et al., 1991; Marco and Stall, 1983). The development of bacteria resistant to antibiotics and chemicals and concomitant reduction in efficacy of these bactericides has sustained interest in developing alternative control strategies to combat bacterial diseases in vegetable crops.

Bacteriophages (phages) are viruses that only invade bacteria. They are generally species or subspecies specific, do not infect nontarget, beneficial bacteria, and also are nontoxic to humans. As early as 1926 (Moore, 1926), bacteriophages were used as a biocontrol agent and were effective in controlling Stewart's disease in corn (Thomas, 1935). Using bacteriophages, Civerolo and Kiel (1969) reduced bacterial spot disease of peach seedlings caused by *Xanthomonas oryzae* by 86% to 100%. However, use of bacteriophages was abandoned because of the emergence of bacterial mutants resistant to the bacteriophages used and the development of successful control measures with antibiotics and other chemicals. An approach that uses a mixture of host-range mutant (h-mutant) bacteriophages was developed to overcome the problems experienced in the past (Jackson, 1989). H-mutant bacteriophages, capable of attacking an extended range of hosts, are spontaneously derived from their wild-type parent bacteriophages, and are so named because they lyse not only parent wild-type bacteria, but also bacteriophage-resistant mutants originating from parent bacteria (Davis et al., 1973).

The objective of this study was to evaluate an h-mutant mixture of bacteriophages as a biocontrol agent for *Xcv.* on tomato seedlings grown under greenhouse conditions and on staked tomatoes in field production. Bacteriophages were compared to the standard commercial control strategy that utilizes bactericides (copper/mancozeb) for control of this economically important disease.

Materials and Methods

Seedling stage (Fall 1997, 1998). 'Sunbeam' tomato seeds were sown 6 July 1997 or 7 July 1998 into a soilless mix in styrofoam flats (43 cm^3 cell size; Speedling, Sun City, Fla.), and placed in a fiberglass production house [30 to 35 °C (day)/20 to 25 °C (night) temperatures] to germinate and reach the two-to-three leaf stage (≈2 weeks) before initiation of treatments. Seedlings were watered as needed with overhead irrigation. The treatments were as follows: 1) control (water only); 2) copper/mancozeb [a mixture of Kocide 101® (Griffin Corp., Valdosta, Ga.) at 2.08 $g \cdot L^{-1}$ a.i. and Manzate 200 DF® (DuPont, Wilmington, Del.) at 1.05 $g \cdot L^{-1}$ a.i.] applied every 5 d; or 3) a mixture of four h-mutant bacteriophages (Agriphage; Agriphi, Logan, Utah) diluted to a final concentration of 10^8 plaque-forming units per mL (pfu/mL) applied in each irrigation event. The flats were treated for 3 weeks before seedlings were transplanted to the field. The experimental design was a randomized complete block with four replications.

One week after initiating treatments, the center plant in each flat was inoculated with *Xcv.* by infiltrating a suspension containing ≈10^8 cfu/mL of a mixture of two *Xcv.* tomato race 1 (T1) and two tomato race 3 (T3) strains. Disease incidence (percentage of plants exhibiting bacterial spot symptoms) was assessed for each flat 1 day prior to transplanting. Lesions from each treatment were sampled and the isolated bacteria were confirmed to be *Xcv.*

Field stage (Fall 1997, 1998). Thirty seedlings, chosen at random from each flat, were transplanted into field plots on 15 Aug. 1997 or 19 Aug. 1998. The field plots consisted of three raised beds spaced on 152-cm centers with plants spaced 45-cm apart in the row. The beds were 20-cm high × 76-cm wide × 630-cm long, and were previously fumigated with 67% methyl bromide : 33% chloropicrin at 239 $kg \cdot ha^{-1}$, and covered with white-on-black (white side up) polyethylene mulch. Standard fertilization and seepage irrigation practices were used, and all plots were sprayed weekly with the standard fungicide and insecticide

Received for publication 26 July 1999. Accepted for publication 10 Dec. 1999. Florida Agricultural Experiment Station Journal Series no. R-07021. We thank the Florida Tomato Committee and the USDA-SBIR for partial funding of this research. We also thank Rick Kelly for his tireless technical assistance and contributions to this research. Use of trade names does not imply endorsement of products or criticism of similar ones not mentioned. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Assistant In Plant Pathology/Horticulture.

[2]Professor of Plant Pathology. Current address: Dept. of Plant Pathology, Univ. of Florida, 1439 Fifield Hall, Gainesville, FL 32611.

[3]Professor of Floriculture. To whom reprint requests should be addressed. E-mail address: brenth@nersp.nerdc.ufl.edu

[4]Research Scientist, Agriphi, P.O. Box 4296, Logan, UT 84323.

treatments for foliar fungal pathogens and insect pests (Hochmuth et al., 1988).

Each field plot consisted of three rows of 15 plants each. Field treatments were initiated immediately following transplanting. Seedlings from each of the greenhouse treatments were subdivided into the following three field treatments: 1) untreated control; 2) copper/mancozeb (same rate as above) applied as foliar sprays every 4–5 d for a total of 18 (1997) or 24 (1998) applications; or 3) bacteriophages applied as foliar sprays twice weekly (same rate as above) for a total of 29 applications in both 1997 and 1998. Bacteriophages were applied at or before daybreak to minimize UV degradation. Bacteriophage and copper/mancozeb treatments were applied by spraying to runoff using a 276 kPa compressed CO_2 sprayer until full coverage was obtained.

Disease severity ratings were recorded using two different procedures. The first involved determining lesion numbers on 30 leaflets collected from separate leaves. This procedure was performed on 6 Oct. during the Fall 1997 field season and on 15 Oct. during the Fall 1998 field season. The second disease severity rating consisted of assessing the percentage of defoliation on three dates using the Horsfall-Barratt scale (Horsfall and Barratt, 1945) and calculating the area under the disease progress curve (AUDPC, Shaner and Finney, 1977) for each plant. This area was used for statistical analysis. Along with disease severity ratings, plant vigor was rated twice prior to harvesting fruit (10 Sept. and 15 Oct. 1997; 12 and 30 Oct. 1998) on a scale ranging from 0–7, where 0 represented a sparse plant canopy, necrotic leaf spots on most of the leaves on each plant, and severe epinasty of new growth; and 7 represented a uniform plant canopy, few or no necrotic leaf spots on most of the leaves of each plant, and lack of epinasty on new growth. Marketable fruit was harvested from 10 plants from each plot on 23 Oct.; 4 and 10 Nov. (Fall 1997); and 3 and 17 Nov. (Fall 1998). The fruit were graded according to size and then weighed.

The 3 (greenhouse) × 3 (field) factorial treatments were replicated four times in a split-plot design (main plots = greenhouse treatments) (Fall 1997), or a randomized complete-block design (Fall 1998). Significance of the main factors and interactions were determined by analysis of variance (ANOVA) procedures using SAS (SAS Inst., Cary, N.C.). Where significant ($P \leq 0.05$) main treatment effects occurred, differences between means were determined by Duncan's multiple range test ($P \leq 0.05$).

Results

Seedling stage

Fall 1997. Seedlings treated with either copper/mancozeb or bacteriophages displayed significantly lower disease incidence (5.5% and 0.5%, respectively) than did the water control (40.5%), (Table 1). The copper/mancozeb and bacteriophage treatments did not differ significantly from each other.

Fall 1998. The average incidence of bacterial spot on greenhouse grown transplants was 17.4% in the control flats vs. 5.5% for plants treated with copper-mancozeb or 2.7% for plants treated with bacteriophages; however, differences were not significant at $P \leq 0.05$.

Field stage

There were no interactions between greenhouse and field treatments for either season in these 3 × 3 factorial-designed experiments, so the main effects will be discussed.

Fall 1997. The greenhouse treatments had an effect on AUDPC ratings in the field, as seedlings treated with bacteriophages displayed a significantly lower AUDPC value than either the control or seedlings treated with bactericides (Table 2). However, greenhouse treatments had no effect on either lesion counts or plant vigor ratings. Field treatments affected both AUDPC values and plant vigor ratings but not lesion counts. Plants in field plots treated with copper/mancozeb or bacteriophages had significantly lower AUDPC values than did control plants. Plants treated with bacteriophages had significantly higher plant vigor ratings than did control plants, which, in turn, had higher ratings than did plants treated with copper/mancozeb.

Seedlings treated with bacteriophages in the greenhouse produced more extra-large fruit at harvest than did control plants, whereas the copper/mancozeb treatments resulted in plants with intermediate numbers and weight of extra large fruit. For the field treatments, number and weight of extra-large fruit were increased significantly by the bacteriophage treatment, but not by copper/mancozeb. The numbers or weights of large or medium size fruit were not affected by either greenhouse or field treatments.

Fall 1998. Greenhouse and field treatments influenced AUDPC ratings of field plants. Treatment of seedlings with bacteriophages in the greenhouse significantly reduced AUDPC values, but treatment with copper/mancozeb did not (Table 2). Greenhouse treatments did not influence lesion counts or plant vigor ratings in the field. However, field-bacteriophage treatments significantly reduced lesion count number and stimulated growth while copper/mancozeb treatments had an intermediate effect.

Greenhouse treatments did not influence fruit numbers or weights. However, the number and weight of extra-large and large fruit were greater with field-bacteriophage treatments but not with copper/mancozeb.

Table 1. Effects of bacteriophages and chemical bactericides on incidence of bacterial spot on tomato seedlings in the greenhouse.

	Bacterial spot incidence[z] (%)	
Treatment	1997	1998
Control	40.5 a[y]	17.4 a
Copper/Mancozeb	5.5 b	5.5 a
Bacteriophage	0.9 b	2.7 a

[z]Percentage of seedlings with bacterial spot immediately prior to field transplantation.
[y]Mean separation within columns by Duncan's multiple range test, $P \leq 0.05$.

Discussion

In this study, applications of bacteriophages consistently reduced incidence and severity of bacterial spot disease caused by *Xcv*. This resulted in healthier, more vigorous plants that produced significantly better yields than did control plants or plants treated with a standard copper/mancozeb bactericide. Thus, bacteriophages are a very promising alternative to copper bactericides and may serve to significantly reduce economic losses incurred by tomato growers due to the bacterial spot pathogen, *Xcv*. The probable environmental benefits of reducing copper- containing pesticides suggest another distinct advantage of this method.

The effectiveness of conventional bactericides (i.e., copper compounds and streptomycin) used for controlling bacterial diseases has been limited as a result of the common occurrence of *Xcv*. strains resistant to streptomycin and copper (Marco and Stall, 1983; Thayer and Stall, 1962). With the high probability of *Xcv*. strains developing resistance or being resistant to the various bactericides now existing, bacteriophage mixtures containing several h-mutants offer an excellent strategy to maintain effective control. *Xcv*. strains may develop resistance to wild-type bacteriophages; however, the likelihood of *Xcv*. strains developing resistance to multiple h-mutants is remote. Furthermore, if resistant *Xcv*. strains developed, new virulent bacteriophages could be identified to control those strains.

Future studies will examine the effectiveness of compounds that may extend the life of bacteriophages in the field, thus eliminating the need for early morning spraying, reducing application frequencies and overall costs. The possibilities of using h-mutant bacteriophages for preventing bacterial diseases in other crops such as geranium (*Pelargonium* ×*hortorum* L.H. Bail.) and poinsettia (*Euphorbia pulcherrima* Willd.) production are also under investigation (Harbaugh et al., 1999).

Literature Cited

Civerolo, E.L. and H.L. Kiel. 1969. Inhibition of bacterial spot of peach foliage by *Xanthomonas prunie* bacteriophage. Phytopathology 59:1966–1977.

Davis, B.D., R. Dulbecco, H.N. Eisen, H.S. Ginsburg, W.B. Wood, and M. McCarty. 1973. Microbiology. 2nd ed. Harper and Rowe, Hagerstown, Md.

Harbaugh, B.K., J.B. Jones, L.E. Jackson, G. Somodi, and J.E. Flaherty. Biological control of bacterial blight of geranium with h-mutant bacteriophages. HortScience 33:519. (Abstr.)

Hochmuth, G.J., D.N. Maynard, and M. Sherman. 1988. Tomato production guide for Florida. Florida Coop. Ext. Circ. 98C.

Horsfall, J.G. and R.W. Barratt. 1945. An improved system for measuring plant disease. Phytopathology 35:655.

Jackson, L.E. 1989. Bacteriophage prevention and

Table 2. Main effects of h-mutant bacteriophage and chemical bactericides applied during greenhouse production of seedlings or applied during field production of staked tomatoes on disease incidence and severity and fruiting parameters of 'Sunbeam' tomatoes, Fall 1997 and Fall 1998. Interactive effects of greenhouse × field treatments were not significantly different ($P \leq 0.05$). Main effect means for seedling treatments are averaged over field treatments, and main effect means for field treatments are averaged over seedling treatments.

	Plant health				Fruit no. and yield					
					Extra large		Large		Medium	
Treatment	AUDPC[z]	Incidence[y]	Vigor I[x]	Vigor II	No.	Total wt (kg)	No.	Total wt (kg)	No.	Total wt (kg)
					Fall 1997					
					Greenhouse effects					
Control	113.9 a[w]	33.8 a	4.3 a	3.1 a	115 b	21.4 b	63 a	9.1a	30 a	3.2 a
Copper/Manc.	105.3 a	29.5 a	4.0 a	3.6 a	131 ab	25.5 ab	69 a	10.0 a	27 a	3.2 a
Bacteriophages	82.5 b	32.3 a	3.8 a	3.1 a	143 a	27.3 a	71 a	10.0 a	27 a	3.2 a
					Field effects					
Control	113.6 a	32.4 a	2.8 b	3.6 b	126 b	24.1 b	67 a	9.5 a	32 a	3.6 a
Copper/Manc.	94.4 b	35.5 a	1.6 c	2.7 c	119 b	22.3 b	65 a	9.1 a	25 a	2.7 a
Bacteriophages	93.7 b	28.2 a	4.5 a	4.7 a	143 a	27.7 a	72 a	10.5 a	27 a	3.2 a
					Fall 1998					
					Greenhouse effects					
Control	192.2 a	16.2 a	4.2 a	5.4 a	126 a	25.5 a	65 a	9.5 a	35 a	4.1 a
Copper/Manc.	191.6 a	10.9 a	4.1 a	5.7 a	134 a	28.2 a	63 a	9.1 a	30 a	3.2 a
Bacteriophages	175.8 b	10.8 a	3.9 a	5.4 a	137 a	30.5 a	68 a	10.0 a	32 a	4.1 a
					Field effects					
Control	201.9 a	22.3 a	3.5 a	4.9 c	123 b	24.1 b	58 b	8.6 b	32 a	3.6 a
Copper/Manc.	189.6 a	13.4 b	3.8 a	5.5 b	121 b	26.8 b	62 ab	9.1 b	30 a	3.6 a
Bacteriophages	168.0 b	6.4 c	4.8 a	6.0 a	154 a	33.2 a	76 a	10.9 a	34 a	4.1 a

[z]AUDPC = area under the disease progress curve.
[y]Average number of bacterial spot lesions per leaflet based on sample of 30 leaves per plot.
[x]Scale from 0–7: 0 = sparse plant canopy, necrotic leaf spots on most of the leaves on each plant, severe epinasty of new growth; 7= uniform plant canopy, few or no necrotic leaf spots on most of the leaves on each plant, lack of epinasty of new growth.
[w]Mean separation within columns for greenhouse or field effects by Tukey's Studentized (HSD) range test, $P \leq 0.05$.

control of harmful plant bacteria. U.S. Patent No. 4,828,999.

Jones, J.B. and J.P. Jones. 1985. The effect of bactericides, tank mixing time and spray schedule on bacterial leaf spot of tomato. Proc. Fla. State Hort. Soc. 98:244–247.

Jones, J.B. and J.W. Scott. 1986. Hypersensitive response in tomato to *Xanthomonas campestris* pv. *vesicatoria*. Plant Dis. 70:337–339.

Jones, J.B., R.E. Stall, J.W. Scott, G.C. Somodi, H. Bouzar, and N.C. Hodge. 1995. A third tomato race of *Xanthomonas campestris* pv. *vesicatoria*. Plant Dis. 79:395–398.

Jones, J.B., S.S. Woltz, J.P. Jones, and K.L. Portier. 1991. Population dynamics of *Xanthomonas campestris* pv. *vesicatoria* on tomato leaflets treated with copper bactericides. Phytopathology 81:714–719.

Kousik, C.S. and D.F. Ritchie. 1996. Race shifts in *Xanthomonas campestris* pv. *vesicatoria* within a season in field-grown pepper. Phytopathology 86:952–958.

Marco, G.M. and R.E. Stall. 1983. Control of bacterial spot of pepper initiated by strains of *Xanthomonas campestris* pv. *vesicatoria* that differ in sensitivity to copper. Plant Dis. 67:779–781.

Moore, E.S. 1926. D'Herelle's bacteriophage in relation to plant parasites. South Afr. J. Sci. 23:306.

Pohronezny, K., R.E. Stall, B.I. Canteros, M. Kegley, L.E. Datnoff, and R. Subramanya. 1992. Sudden shift in the prevalent race of *Xanthomonas campestris* pv. *vesicatoria* in pepper fields in southern Florida. Plant Dis. 76:118–120.

Pohronezny, K., and R.B. Volin. 1983. The effect of bacterial spot on yield and quality of fresh market tomatoes. HortScience 18:69–70.

Scott, J.W. and J.B. Jones. 1986. Sources of resistance to bacterial spot [*Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye] in tomato. HortScience 21:304–306.

Shaner, G. and R.E. Finney. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051–1056.

Thomas, R.C. 1935. A bacteriophage in relation to Stewart's disease of corn. Phytopathology 25:371–372.

Thayer, P.L. and R.E. Stall. 1962. Streptomycin resistance of the bacterial spot pathogen and control with streptomycin. Plant Dis. Rptr. 46:389–392.