

Comparison of Biological and Chemical Treatments for Control of Bacterial Speck of Tomato Under Field Conditions in Morocco

J. E. COLIN and Z. CHAFIK, Institut Agronomique et Veterinaire Hassan II, Complexe Horticole d'Agadir, B.P. 438, Agadir, Morocco

ABSTRACT

Colin, J. E., and Chafik, Z. 1986. Comparison of biological and chemical treatments for control of bacterial speck of tomato under field conditions in Morocco. *Plant Disease* 70: 1048-1050.

Bacterial speck of tomato caused by *Pseudomonas syringae* pv. *tomato* was partially controlled by biological and chemical treatments. The effects of *P. syringae* pv. *tomato* infection and of control treatments were significant on percentage of reject fruit (total and specked) but not on total fruit yield and flower drop. Weekly applications of two antagonistic pseudomonads gave a level of protection equal to that of copper compounds and had a slightly greater residual effect.

Bacterial speck of tomato (*Lycopersicon esculentum* L.) caused by *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye, & Wilkie (*P. s.* pv. *tomato*) was first reported in Morocco in

winter 1980 (1). Under field conditions, the disease reduced yield but had its primary effect on fruit quality (2). Apart from cultural practices such as modification of irrigation practices to limit disease loss (10) or use of copper compounds, alone or in combination with fungicides, in protective treatments (5), there is no effective way to control bacterial speck of tomato.

Several isolates of fluorescent *Pseudomonas* were found to be antagonistic in vitro to phytopathogenic species of *Erwinia*, *Corynebacterium*, *Xantho-*

monas, and *Pseudomonas*, and two of these antagonistic *Pseudomonas* were able to decrease the number of foliar lesions induced by *P. s.* pv. *tomato* on tomato leaves under laboratory conditions (4).

The purpose of this investigation was to confirm on mature plants under field conditions the potential of antagonistic fluorescent pseudomonads to protect tomato plants against bacterial speck disease.

MATERIALS AND METHODS

Bacterial pathogen and antagonists.

Strain 367 of *P. s.* pv. *tomato* was isolated in Morocco (1). Strains 208 and 381 of fluorescent *Pseudomonas* were isolated from epiphytic microorganism populations of tomato and selected for their high level of antagonistic activity (4).

All bacteria were grown on King's medium B (9) and produced in Roux bottles. Inocula of antagonists and pathogen were prepared by diluting

Accepted for publication 22 March 1986.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

©1986 The American Phytopathological Society

1048 Plant Disease/Vol. 70 No. 11

bacterial suspensions from 48-hr cultures in tap water to a final concentration of 10^9 colony-forming units per milliliter.

Chemical control treatments. Three bactericides were compared with the antagonistic bacterial treatments. The chemicals and their concentrations in tap water were: ammoniacal copper sulfate (COPAC E, BASF Aktiengesellschaft) at 0.15 g/L cupric metal, cupric oxychloride (COBOX 50, BASF Aktiengesellschaft) at 2.11 g/L cupric metal, and streptomycin sulfate (Merck, art. 10117) at 500 ppm.

Field application. Antagonistic bacterial and chemical suspensions were applied with a hand sprayer to cover entire plant surfaces to runoff. Protective treatments began on 2 April at first cluster formation with most flowers open and were performed at either 1- or 2-wk intervals. Except for uninoculated controls, all plants, protected (weekly and biweekly) or unprotected, were inoculated weekly with a *P. s. pv. tomato* suspension. *P. s. pv. tomato* inoculum was applied 1 hr after protective treatments. All sprayings were stopped after formation of the fourth blossom cluster, which was the last cluster treated.

The trial was performed in spring 1984 in the Massa Plain south of Agadir, Morocco. Weather conditions during the trial were 24 and 12.6 C for maximum and minimum mean temperatures, respectively, 77% mean relative humidity, and 12.7 mm of recorded rainfall.

Tomato cultivar Carmello was sown and transplanted in sterile soil. At the three-leaf stage, plants were planted in the field on 2 February. One stem was kept, and plants were topped above the fourth cluster.

Twelve treatments were arranged in a randomized complete block design with four replicates. Planting was done in double rows. Each block comprised one and a half rows. Each plot contained 5×2 plants separated from the adjacent plot by 7×2 plants without inoculation or treatment. Plants were 0.4 m apart in the double row and interrow spacing was 1.4 m.

The crop was drip-irrigated, but sprinkler irrigation was applied each week for 1 hr on windless mornings to promote disease development 3 days after inoculation. Fruit harvesting began on 18 May at the color-turning stage and lasted for 5 wk.

Bacterial speck symptoms were monitored by observing all leaves of inoculated and uninoculated plants. Effects of the disease and the protective treatments were estimated by fruit yield of the four clusters (10 plants per plot). At each harvest, the fruits were examined, counted, and weighed to determine 1) total yield (grams per plant); 2) total rejects of abnormal fruits, including bacterial specked fruits (weight percentage of total yield); and 3) rejects caused by bacterial speck symptom only

(*P. s. pv. syringae* rejects [weight percentage of the total yield]). The percentage of flower drop was calculated (at the last harvest on the four clusters) by totaling fruits and flower scars.

Data were subjected to mean comparisons with the Newman-Keuls method at $P = 0.05$ (6).

RESULTS

Lesions on leaves appeared a few days after the first inoculation on all inoculated plants. Lesion numbers increased too rapidly to apply a disease index and establish a disease rating. The dry weather conditions were, however, unfavorable for disease spread, and no symptoms appeared on uninoculated plants except on those adjacent to inoculated plants.

Results of total yields and of rejects, specked fruits, and flower drop rates are reported in Table 1.

Total yields were unaffected by the phytopathogenic bacteria, as shown in the nonsignificant differences between treatments, even between uninoculated and inoculated checks. *P. s. pv. tomato* rejects, expressed as a weight percentage, and numbers of specked fruit are highly correlated (0.99) because of the nonsignificant differences in flower drop in this trial. Fruit size was not modified by the *P. s. pv. tomato* infection relative to the control plants. *P. s. pv. tomato* rejects, which comprised all specked fruits, a specific symptom of the pathogen, constituted a part of total rejects (unmarketable fruits). Other criteria for unmarketability were other diseases, misshapen fruits, and bird damage. *P. s. pv. tomato* rejects and total rejects are highly correlated ($r = 0.98$).

For weekly protective treatments, best results were obtained with streptomycin sulfate, but the two antagonistic bacteria also significantly reduced bacterial speck fruit symptoms and gave a level of protection similar to that of two copper compounds.

Biweekly protective treatments were also effective. In addition, the antagonistic bacteria appeared to have a slightly better residual activity than copper compounds and protected as effectively as streptomycin sulfate, although the differences between the treatments were not statistically significant. Like total yield, flower drop rates were not significantly affected by *P. s. pv. tomato* inoculation and protective treatments. Except for the biweekly ammoniacal copper sulfate treatment, all protective treatments gave flower drop rates slightly lower than those of the inoculated controls. The slight differences observed were nevertheless correlated with the relative effectiveness of the protective treatment.

DISCUSSION

Bacterial speck disease of tomato did not always affect tomato yield, even with severe fruit speck development (5). It was previously shown that under Moroccan conditions, bacterial speck disease affected tomato yield under sprinkler but not under drip irrigation and that *P. s. pv. tomato* rejects from inoculated plants were markedly higher under sprinkler than under drip irrigation (2). Under these same conditions, there was a highly significant difference of flower drop between inoculated and uninoculated plants.

In the present work, to ensure disease

Table 1. Effects of biological and chemical control treatments on total yield, total and *Pseudomonas syringae* pv. *tomato* rejects^w and flower drop of Carmello tomato inoculated with *P. s. pv. tomato*

| Protective treatment | Total yield (g/plant) | Total rejects (% of total yield) | <i>P. s. pv. syringae</i> rejects (% of total yield) | Flower drop (% of total formed flowers) |
|---------------------------------------|-----------------------|----------------------------------|--|---|
| Uninoculated plants | 2,881 ^x | 18.2 a ^y | 2.2 a | 32.9 |
| <i>P. s. pv. syringae</i> control | 3,140 | 66.0 e | 59.5 d | 35.9 |
| Weekly protective treatment | | | | |
| <i>Pseudomonas</i> (208) | 2,972 | 26.5 abc | 16.3 b | 31.6 |
| <i>Pseudomonas</i> (381) | 2,995 | 32.8 cd | 16.8 b | 32.3 |
| Ammoniacal copper sulfate | 3,025 | 24.3 ab | 13.4 b | 29.9 |
| Cupric oxychloride | 3,064 | 31.4 bcd | 15.2 b | 34.1 |
| Streptomycin sulfate | 3,202 | 24.3 ab | 9.7 b | 29.1 |
| Biweekly protective treatment | | | | |
| <i>Pseudomonas</i> (208) | 3,045 | 34.6 cd | 25.0 c | 34.8 |
| <i>Pseudomonas</i> (381) | 3,096 | 36.8 cd | 23.4 c | 30.3 |
| Ammoniacal copper sulfate | 3,213 | 41.7 d | 27.2 c | 37.2 |
| Cupric oxychloride | 3,099 | 41.2 d | 27.7 c | 32.4 |
| Streptomycin sulfate | 3,337 | 33.2 cd | 23.2 c | 28.4 |
| Statistical significance ^z | NS | ** | ** | NS |

^wIncluded fruits with bacterial speck symptoms only.

^x Each number is the result of four replicates of 10 plants observed.

^y Within each column, numbers followed by the same letter are not significantly different according to the Newman-Keuls method ($P = 0.05$).

^z NS = nonsignificant difference between treatments and ** = significant at $P = 0.01$.

development in the field trials, *P. s. pv. tomato* was applied at high concentration and at weekly intervals to inoculate each cluster at its most susceptible state of development (open flowers to fruit ≤ 3 cm) as shown (8) and explained (7) for *P. s. pv. tomato*. We observed a high *P. s. pv. tomato*-reject rate associated with nonsignificant differences in flower drop rate between all treatments. On the other hand, weather conditions were unfavorable for disease development, even with a high infection pressure. Nevertheless, with this artificially high infection pressure, the ability of antagonistic fluorescent *Pseudomonas* spp. to partially control tomato speck disease was demonstrated and so confirmed our previous results on young plants (4). The slightly better residual activity of antagonistic bacteria relative to that of copper compounds may be explained by their capacity to maintain a residual epiphytic population on tomato

leaves (J. E. Colin, *unpublished*).

Although it would be premature to expect immediate applications of antagonistic bacteria in biological control methods, our results and previous reports (3) indicate the potential of this method of disease control.

ACKNOWLEDGMENTS

This work was supported by the Belgo-Moroccan cooperation "Defense des Vegetaux" project financed by the Administration Generale de la Cooperation au Developpement (AGCD), Brussels, Belgium.

LITERATURE CITED

1. Colin, J. 1983. Presence de *Pseudomonas syringae* pv. *tomato* (Okabe), Young *et al.* dans les cultures de tomate au Maroc. *Parasitica* 39:183-185.
2. Colin, J., Gerard, M., and Laabari, H. 1984. Influence du type d'irrigation sur la moucheture bacterienne chez la tomate au Maroc. *Parasitica* 40:3-12.
3. Colin, J., and Pussemier, L. 1985. Emploi des antagonistes bacteriens en Phytopathologie—

- cas des *Pseudomonas* fluorescents. *Rev. Agric.* 38:211-219.
4. Colin, J., Pussemier, L., and Diouf, A. M. 1984. Demonstration et application de l'antagonisme du a des *Pseudomonas* fluorescents sur des bacteries phytopathogenes. *Meded. Fac. Landbouwwet. Rijksuniv. Gent* 49:587-596.
 5. Conlin, K. C., and McCarter, S. M. 1983. Effectiveness of selected chemicals in inhibiting *Pseudomonas syringae* pv. *tomato* in vitro and in controlling bacterial speck. *Plant Dis.* 67:639-644.
 6. Dagnelie, P. 1970. *Theorie et Methodes Statistiques*. Vol. 2. J. Duculot, ed. Gembloux, Belgium. 451 pp.
 7. Getz, S., Fulbright, D. W., and Stephens, C. T. 1983. Scanning electron microscopy of infection sites and lesion development on tomato fruit infected with *Pseudomonas syringae* pv. *tomato*. *Phytopathology* 73:39-43.
 8. Getz, S., Stephens, C. T., and Fulbright, D. W. 1983. Influence of development stage on susceptibility of tomato fruit to *Pseudomonas syringae* pv. *tomato*. *Phytopathology* 73:36-38.
 9. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *J. Lab. Med.* 44:301-307.
 10. Rotem, J., and Palti, J. 1969. Irrigation and plant diseases. *Annu. Rev. Phytopathol.* 7:267-288.