Assaying Bactericide Efficacy Against Pseudomonads on Bean Leaf Surfaces

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ABSTRACT

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Populations of a pathogenic strain of *Pseudomonas syringae* pv. *phaseolicola* that had been artificially established on greenhouse-grown pinto bean plants (*Phaseolus vulgaris*) were exposed to several formulations and rates of copper-based bactericides under controlled conditions to measure pesticide efficacy. New formulations of cupric hydroxide and mixtures of copper oxychloride significantly ($P < 0.05$) reduced these populations. Naturally occurring populations of epiphytic pseudomonads on bean leaves were significantly ($P < 0.05$) reduced and bean yields were significantly ($P < 0.05$) increased by applications of copper-based pesticides in the field in eastern Colorado. Results obtained by the two procedures were similar. The assay technique and its applications are discussed.

Additional keywords: bacterial brown spot, disease control, *Pseudomonas syringae* pv. *syringae*

Halo blight, caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young, Dye, and Wilkie, and bacterial brown spot, caused by *P. s.* pv. *syringae* van Hall, have become ubiquitous diseases of dry beans (*Phaseolus vulgaris* L.) in Colorado and surrounding regions during the past 5 yr. These diseases may cause 25% or greater yield loss, especially if there is an abundant carryover of infested bean crop debris and if the weather is moderately cool and moist in the spring and early summer (7,13). Both pathogens can survive and multiply as epiphytes on volunteer and commercial dry bean plants throughout northeastern Colorado (7) and other regions (3,5,6,8,15).

Control measures include using a 2- to 3-yr crop rotation, incorporating bean debris into the soil to reduce survival of inoculum, avoiding early-season plantings to minimize exposure to overwintering inoculum of *P. s.* pv. *phaseolicola* and *P. s.* pv. *syringae* while not planting so late that plants are exposed to rust (*Uromyces appendiculatus* (Pers. ex Pers.) Unger), and planting disease-resistant cultivars. Also, protectant bactericides are recommended for susceptible cultivars if infection is likely to occur before or during bloom or early pod formation, and if weather conditions favor the early establishment and continued development of either bacterial pathogen (10,12,13,17). Although copper-based bactericides such as cupric hydroxide, copper oxychloride, copper sulfate, and copper ammonium carbonate have consistently reduced halo blight incidence, yield increases have been inconsistent in field studies (1,2,7,9,11,16).

This paper describes a technique to evaluate efficacy of bactericides and presents efficacy data for copper bactericides evaluated using this technique on greenhouse- and field-grown plants. A preliminary report has been published (14).

MATERIALS AND METHODS

Assay on greenhouse-grown plants. Seeds of pinto beans (*P. vulgaris* 'Olathe') were planted weekly in the greenhouse; 10–14 days later, the seedlings were sprayed with a suspension of bacterial cells. Six seeds were planted per 15-cm pot, which was placed on a heated germination mat at 25 C. Seven days later, seedlings were thinned to three per pot.

A pathogenic strain of *P. s.* pv. *phaseolicola* (PSP 87-B45) that had been recovered from an infected Sacramento Light Red Kidney bean plant found near

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Holyoke, Colorado, in 1987 was cultured on King's medium B (4) for 48 hr. The cells were then suspended in sterile phosphate buffer (0.01 M) and adjusted to a concentration of approximately 10^8 cfu/ml using a spectrophotometer (70% transmittance at 640 nm). Bacterial cells were misted onto seedling leaves (primarily the adaxial surface) at the rate of 935 L/ha within a closed chamber using a CO_2 pressurized sprayer system and a nozzle pressure of 220 kPa. Leaves were air-dried for 30–60 min, then placed into a dark Percival Dew Chamber Model I-35 for 12 hr. This was followed by a 24-hr dry period at 22–26 C.

Plants were then misted in an enclosed chamber (75 × 75 × 75 cm) with a copper bactericide at the rate of 935 L/ha, dried for 30 min, and misted again with 935 L/ha of water to simulate deposition of dew and to allow redistribution of the bactericide. The leaves were dried for 1–2 hr, then 18 disks 3.5 cm^2 in diameter (six each from three plants per pot = one rep) were removed with a cork borer and bulked for enumeration of pseudomonads according to the modified dilution plate method of Legard and Schwartz (7).

Copper treatments included cupric hydroxide (Kocide 606, 3.1 L/ha; Agtrol NF, 3.1 L/ha; Champion Fl, 3.1 L/ha; Champ, 3.9 L/ha), copper sulfate (Copper Power, 3.1 L/ha), and copper oxychloride (Bravo C/M, 1.1, 2.2, 3.4, 4.5, and 6.7 kg/ha; SDS 64216, 4.5 and 6.7 kg/ha). Each copper product and rate except Champion Fl was compared with an untreated control in at least three replicated assays. A completely randomized experimental design with four replications was used for each experiment.

Field efficacy trials. All products and most rates used on the greenhouse-grown plants were also tested in the field at two sites in 1988. Epiphytic populations were monitored only on control plants and plants treated with three products (Agtrol NF, Champ, and Bravo C/M) that had been shown to be effective on greenhouse-grown plants. At site 1 (Bay Farm Research Facility, Fort Collins, Colorado), which had not been planted to beans for 3 yr, pinto bean cultivar U.I. 114 was planted on 25 May. Bactericides were applied at the rate of 234 L of water per hectare with a CO_2 backpack sprayer at 220 kPa pressure on 6 July (full bloom) and 22 July (early pod set). The site was furrow-irrigated as needed for adequate plant growth. Each two-row (75-cm) plot was 4.5 m^2 (1.5 m wide × 3 m long) and was adjacent to a row of beans that had been inoculated with a 1 × 10^8 suspension of *P. s.* pv. *phaseolicola* cells on 29 June. Healthy appearing trifoliolate leaves (two-thirds to fully expanded) were collected from inoculated plants on 8 July and from bactericide-treated and untreated control plants on 22 July, and the number of epiphytic bacteria were enumerated as described previously (7).

Site 2, a commercial field near Bethune, Colorado, was planted with pinto bean cultivar U.I. 126 on 26 May. Copper treatments were applied as described for site 1 on 20 June (prebloom), 30 June (early bloom), 13 July (pod set), and 27 July (early pod fill). The field had been planted to dry beans during the previous three consecutive years. Volunteer beans distributed throughout the field showed halo blight and bacterial brown spot lesions during the entire growing season. Each four-row (75-cm) plot was 22.5 m^2 (3 m wide × 7.5 m long) and was irrigated with an overhead sprinkler as needed for adequate plant growth. Symptomless trifoliolate leaves that were two-thirds to fully expanded were collected from volunteer bean plants (17 June) and new-crop plants (17 June, 1 July, 15 July, 29 July) and assayed for epiphytic populations. Newly opened blossoms and symptomless flat pods were also collected from new-crop plants on 15 July and 29 July, respectively. Samples were processed and epiphytic pseudomonad populations enumerated (7). A randomized complete block design with four replications per treatment was used for each field experiment. Plants from the plots were harvested manually on 17 August and air-dried for 14 days before threshing.

Statistical analyses were made using a one-way analysis of variance, and a standard mean separation test was performed if significant ($P < 0.05$) differences were indicated.

RESULTS

Assay on greenhouse-grown plants. The initial populations of the pathogen were near 1 × 10^5 cfu/cm^2 in the four experiments (Table 1).

Cupric hydroxide and copper sulfate products were compared in the first experiment. Newer formulations of cupric hydroxide (Champ, Agtrol NF) and copper sulfate (Copper Power) significantly ($P < 0.05$) reduced populations of the pathogen compared with those on untreated control plants. In the second experiment, copper oxychloride mixtures and rates were compared with Kocide 606 and the untreated control. Bravo C/M (4.5 and 6.7 kg/ha) significantly ($P < 0.05$) reduced populations of *P. s.* pv. *phaseolicola* compared with the control and Kocide 606, which is an earlier formulation of cupric hydroxide. SDS 64216 and Kocide 606 both significantly reduced populations compared with the control. The most effective treatments in the first and second experiments were compared with Kocide 606 and the control in the third and fourth experiments. Bravo C/M, Champ, and Kocide 606 significantly ($P < 0.05$) reduced populations of *P. s.* pv. *phaseolicola* in both experiments. Bravo C/M was the most effective bactericide included in experiment three; experiment four demonstrated a dosage effect with this bactericide.

Field efficacy trials. At site 1, epiphytic populations of *P. s.* pv. *phaseolicola* were 1 × 10^6 bacteria per leaflet of inoculated plants on 8 July. Populations on uninoculated plants were significantly ($P < 0.05$) reduced by the new copper formulations when assayed on 22 July (Table 2). Hot and dry conditions that occurred during late July and August reduced epiphytic populations below the threshold for detection by the end of July. No infection was observed, and yield data were not collected.

At site 2, 40 leaflets from volunteer beans and 40 from Pinto U.I 126 seedlings were randomly selected throughout the experimental site on 17 June and assayed for epiphytic populations of fluorescent pseudomonads. A population of 1 × 10^6 fluorescent pseudomonads was found on leaflets from both sources. Cultural characteristics and pathogenicity tests of selected isolates indicated that they were primarily *P. s.* pv. *phaseolicola* and *P. s.* pv. *syringae* (7).

Table 1. Comparison of populations of *Pseudomonas syringae* pv. *phaseolicola* on greenhouse-grown plants misted with the bacterium and subsequently sprayed with copper bactericides

Treatment	Rate/ha	Epiphytic population (log_{10})[z]
Experiment 1		
Control	...	5.04 a
Kocide 606	3.1 L	4.71 ab
Champion Fl	3.1 L	4.82 ab
Agtrol NF	3.1 L	4.20 bc
Copper Power	3.1 L	4.15 bc
Champ	3.9 L	3.60 bc
Experiment 2		
Control	...	5.62 a
Kocide 606	3.1 L	4.51 b
SDS 64216	4.5 kg	4.35 b
SDS 64216	6.7 kg	4.21 b
Bravo C/M	4.5 kg	2.91 c
Bravo C/M	6.7 kg	3.04 c
Experiment 3		
Control	...	5.82 a
Kocide 606	3.1 L	4.95 b
Champ	3.9 L	5.08 b
Bravo C/M	4.5 kg	3.65 c
Experiment 4		
Control	...	5.44 a
Kocide 606	3.1 L	4.07 b
Bravo C/M	1.1 kg	3.91 b
Bravo C/M	2.2 kg	3.39 c
Bravo C/M	3.4 kg	3.02 cd
Bravo C/M	4.5 kg	2.74 d

[z]Values are the mean number of cfu/cm^2 on King's medium B for three tests per experiment with each treatment replicated four times. Means followed by the same letter within an experiment are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

Table 2. Comparison of pseudomonad populations on dry bean plants sprayed with copper bactericides in two fields in Colorado in 1988

Spraying			Percent of control		
Plant part	**Date**	**Control**[x]	**Agtrol NF**	**Champ**	**Bravo C/M**
Site 1					
Leaves	22 July	5.80[y,z]	48	69	58
Site 2					
Leaves	1 July	6.53[y,z]	75	85	50
	15 July	6.95[y,z]	98	85	80
	29 July	6.46[z]	84	93	87
Flowers	15 July	5.42[y,z]	92	95	72
Pods	29 July	6.96[z]	100	91	98
Yield (kg/ha)	1 September	2,334[y]	115	116	111

[x] Values are the mean number for each treatment replicated four times at each site.
[y] $P < 0.05$ for orthogonal contrasts between the control and the average of the three bactericide treatments (percent of control).
[z] Fluorescent pseudomonad population (log 10 cfu per leaflet) on King's medium B.

Naturally occurring epiphytic populations of *Pseudomonas* persisted at this site throughout the July sampling period (Table 2). Four sprays with the copper-based bactericides significantly ($P<0.05$) reduced epiphytic populations on foliage and flowers until the middle of July. Epiphytic populations and development of halo blight and bacterial brown spot lesions persisted into late July and early August. Lesions were abundant late in the season in plants in all plots that were sprayed. However, plant canopies were more vigorous and covered more of the interrow area in all plots treated with bactericides than in the control plots. Significant ($P<0.05$) yield increases of 11, 15, and 16% were recorded for plots treated with the newer bactericides Bravo C/M, Agtrol NF, and Champ, respectively.

DISCUSSION

The assay with greenhouse-grown plants was useful for evaluating the effectiveness of bactericides. Populations of *P. s.* pv. *phaseolicola* in the range of $1 \times 10^5/cm^2$ were consistently established on the foliage of plants used in this assay. Similar populations were found occurring naturally on beans in the field. This facilitates using this assay to predict results of field tests with various formulations or rates of copper-based bactericides. Establishment of epiphytic populations before exposure to a bactericide provides a realistic approach to determine the efficacy of bactericides. The results are directly applicable to field situations where bacterial epiphytes are widespread before symptoms occur, including on seedlings of crops such as beans (3,5–8). The assay enabled us to identify promising products for subsequent evaluation under field conditions and to verify field results. It also provided a reliable technique to compare the bactericidal effects of diverse copper products and rates without having to depend on symptom development. This type of assay should be applicable to other bacterial pathogens of beans and other crops.

The studies with *P. s.* pv. *phaseolicola* on greenhouse-grown plants confirmed that copper-based bactericides can significantly reduce epiphytic populations of this pathogen on dry beans (7). The field test indicated similar results with a mixture of *P. s.* pv. *phaseolicola* and *P. s.* pv. *syringae* under the environmental conditions that prevailed during this test. Newer formulations of cupric hydroxide and mixtures of copper oxychloride enhanced control of these epiphytes and improved yield of a vigorously vining cultivar of pinto bean. The yield increase was sufficient to make it economically feasible for producers to apply four timely sprays. A net return of \$125/ha would have been realized based on a cost of \$100/ha for the bactericide and its application and a price of \$0.66/kg of beans. Greater returns might be realized with higher priced market classes or pinto beans with different growth habits that can be integrated within high-input (drilled and direct-combined) production systems in Colorado and surrounding regions.

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