Epidemiology and Control of Bacterial Blight and Canker of Cowpea

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ABSTRACT

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Four major cowpea cultivars grown for processing in Georgia were evaluated in field plots for their susceptibility to bacterial blight and canker, caused by *Xanthomonas campestris* pv. *vignicola*. Analysis of disease progress curves by transforming disease ratings into gompits showed that the cultivars Pinkeye Purple Hull and White Acre were moderately resistant but Mississippi Silver and Coronet were highly susceptible. Sprays of copper and copper + maneb mixtures controlled leaf spot and stem canker symptoms and significantly increased yields. However, high levels of seedborne bacteria were detected in seed harvested from nontreated check plots. Bacteriophage typing was employed to determine the source of inoculum for seedborne bacteria in the different treatments. On the basis of the original distribution of two lysotypes in the field plots, the infestation of seed from copper + maneb treated plots was attributed mainly to interference from inoculum in check plots.

Additional key words: *Vigna unguiculata*

Bacterial blight and canker (BBC) of cowpea (*Vigna unguiculata* (L.) Walp.) caused by *Xanthomonas campestris* pv. *vignicola* (Burkholder) Dye (*X. c. vignicola*) is a seedborne disease (15) that annually reduces yield and lowers quality of cowpeas in Georgia (6) and elsewhere (8,9,14). A significant portion of the crop in Georgia is produced for processing. Because uniform maturation is necessary for mechanical harvesting and consumer preference, only a few cowpea cultivars are grown for processing. Although these cultivars are known to be susceptible to BBC, knowledge was lacking about the disease development and degree of severity in each. Consequently, this study was conducted to evaluate the susceptibility of four major cowpea cultivars to BBC and the epidemiology of the disease in those cultivars.

Measures required to control the disease in cowpeas grown for processing or seed production were also studied. Copper bactericides provided disease control in the field with little effect on seedborne infestation by the bacterium (5). Highly specific bacteriophage typing schemes have been used to identify bacterial pathogens (3,11), to detect sources of inocula (4,10), and to study the ecology and distribution of plant pathogens (12). Using highly specific bacteriophages, we studied the distribution of *X. c. vignicola* in field plots to determine the source of seedborne infestation in plants treated with copper bactericides.

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MATERIALS AND METHODS

Cowpea cultivars Pinkeye Purple Hull, Coronet, Mississippi Silver, and White Acre were planted in field plots on 19 and 27 June 1980 and 10 July 1981. Plots consisting of six replicates in 1980 and four replicates in 1981 of four 15.25-m rows of each cultivar were planted in a randomized complete block design. Treatments were separated by one guard row in 1980 and three guard rows of Pinkeye Purple Hull in 1981. Plants within a row were thinned by hand to a 15-cm spacing. Fertilizer (5-10-15 NPK) at 896 kg/ha and the herbicide trifluralin (1.2 L/ha) were incorporated 10–15 cm into the soil before planting. Overhead sprinkler irrigation was applied as needed and toxaphene 6EC (4.6 L/ha) was used for insect control. Inoculum was introduced into field plots by misting guard rows with a suspension of *X. c. vignicola* strain 80-1 (inoculum density equal to 10^8 cfu/ml) with subsequent natural dissemination into treatment areas.

Inoculum was applied after full expansion of the first trifoliate leaves. During the early stages of disease development, leaf spots were counted in a nondestructive sample of 60 plants in the two center rows of each treatment. Sizes of individual leaf spots and lesion areas were estimated using a disease assessment key constructed with the aid of a dot-counting template (13). Leaf spots and necrotic areas were assigned to four size categories ranging from ≤ 5 cm^2 to ≥ 50 cm^2. In the later stages of disease development, 14–17 days after initial symptom expression, disease ratings were made by the Horsfall-Barratt method (7) and values were converted to percent disease. Total foliar area for each cultivar was determined by counting the total number of leaves of six plants per treatment and by measuring the area of individual leaflets with a dot-counting template. Data were analyzed statistically with a SAS general linear model program (SAS Institute, Cary, NC). All leaf-spot counts were transformed to $\sqrt{\text{count} + 0.5}$ for statistical analysis. The linear and quadratic effects of time were also analyzed.

Control of BBC in Georgia was studied in the field during 1982 and 1983. On the basis of previous experiments, a source of Mississippi Silver was used as a susceptible cultivar. The seed, tested on four occasions in a bioassay (6), was free of seedborne bacteria. Field plots were established and inoculum applied as described previously, except metolachlor (2.24 kg/ha) and permethrin (0.7 L/ha) were applied for weed and insect control, respectively. In 1982, treatments consisted of copper hydroxide (2.24 kg/ha), copper ammonium carbonate (7 L/ha), copper hydroxide (2.24 kg/ha) plus maneb (2.24 kg/ha), copper ammonium carbonate (7 L/ha) plus maneb (2.24 kg/ha), and a check (no bactericidal treatments). Treatments were begun 14 days after planting and were applied on a 7-day interval with a total of seven applications. Yields were taken and seed were tested for the presence of *X. c. vignicola* by methods described previously (6). Disease assessment readings were made as described.

In 1983, the problem of infested seed from apparently healthy plants in treated plots was addressed. Because of no significant differences among chemical treatments in 1982, the number of bactericide treatments was reduced to one, copper ammonium carbonate (7 L/ha) plus maneb (2.24 kg/ha). The same source of Mississippi Silver known to be free of seedborne bacteria was planted in four replicates of twelve 15.25-m rows arranged in a randomized complete block design. Plants in check plots were inoculated with *X. c. vignicola* strain 80-3, designated lysotype A, and plants in treated plots were inoculated with *X. c. vignicola* strain 80-4, designated lysotype B. In preliminary tests, both lysotypes were virulent and equally aggressive. Inoculum was applied as before except to plants in the center four rows of each plot rather than on guard rows as in previous tests. Disease evaluations were made on entire plots, but yields and seed harvest were from six 2-m^2 sites. Seed were hand-harvested from plots and labeled as coming from either diseased or symptomless plants. Any resulting bacteria recovered from seed by infiltrating cowpea leaves in the greenhouse with seed washes (6) were characterized and identified by bacteriophage typing.

Bacteriophages used in this study were isolated at previous times from diseased cowpea leaves with an enrichment technique (1) and stored until needed. After stationary incubation at 22 C for 48 hr, culture suspensions were centrifuged at 4,000 *g* for 30 min. The supernatant was filtered through a membrane filter of 0.45-μm pore diameter and subsequently spotted on lawns of the homologous bacterium used for enrichment. Resulting bacteriophages were "purified" by three successive single-plaque isolations. Ten bacteriophages were assayed against 180 strains of *X. c. vignicola*. Bacteriophages P1 and P6, which differentiate *X. c. vignicola* lysotypes A and B, were selected for use in the field study. These bacteriophages were highly specific, and lytic patterns were unaltered either by their successive passage through the host bacterium or by the bacterium's passage through cowpea.

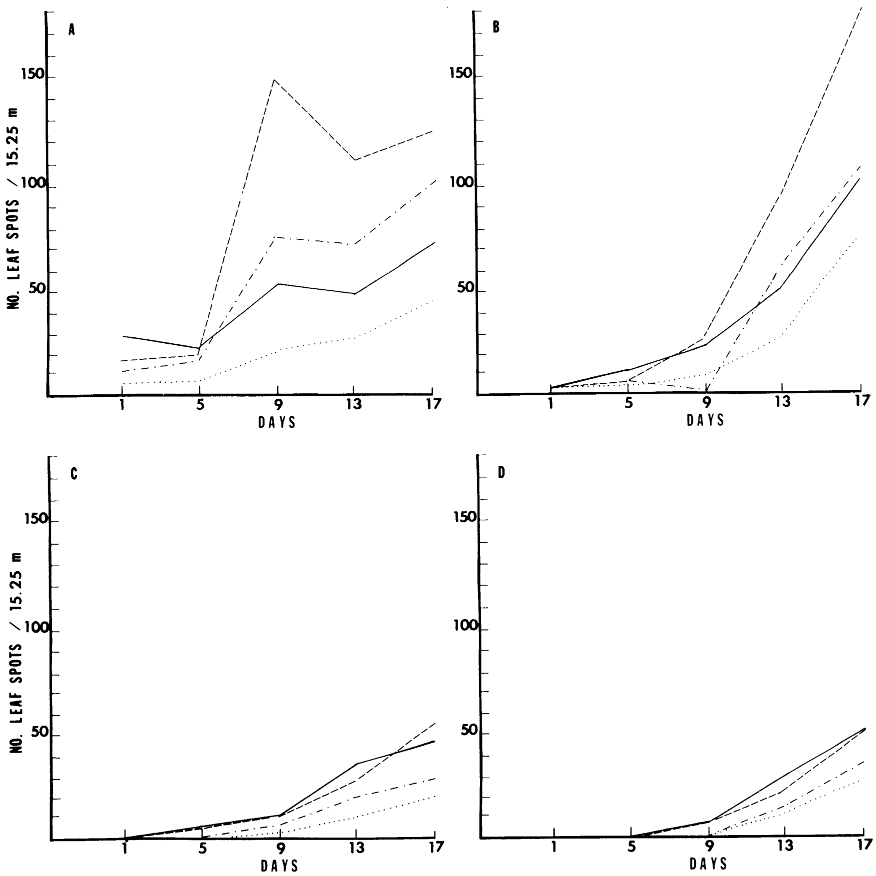

Fig. 1. Numbers of leaf spots on four cowpea cultivars (—— = Coronet, ---- = Mississippi Silver, -·-·- = White Acre, and ····· = Pinkeye Purple Hull) infected with *Xanthomonas campestris* pv. *vignicola* in four size categories: **(A)** ≤5 cm^2, **(B)** 6–20 cm^2, **(C)** 21–49 cm^2, and **(D)** ≥50 cm^2).

Table 1. Number of leaf spots in four size categories on four cultivars of cowpea in the early stages of development of bacterial blight and canker of cowpea

		Number of leaf spots by size per 15.25 m of row				
Year	Cultivar	A (≤5 cm^2)	B (6–20 cm^2)	C (21–49 cm^2)	D (≥50 cm^2)	Total
1980[y]	Mississippi Silver	84 a[z]	62 a	19 a	14 ab	179
	Coronet	44 b	38 ab	18 a	16 a	116
	White Acre	54 ab	34 b	11 b	10 bc	109
	Pinkeye Purple Hull	20 c	22 b	7 b	8 c	57
1981[y]	Mississippi Silver	1,783 a	727 a	73 a	23 a	2,606
	Coronet	898 ab	349 b	29 b	11 a	1,282
	White Acre	753 b	265 b	29 b	6 a	1,053
	Pinkeye Purple Hull	602 b	232 b	19 b	6 a	859

[y] Values represent the mean of six replicates and five sampling dates in 1980 and the mean of four replicates and two sampling dates in 1981.

[z] Data were transformed before analysis to $\sqrt{\text{no. of leaf spots} + 0.5}$. Values within a column for an individual year followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

RESULTS

In 1980, during the early stages of disease development, leaf spots were counted and categorized by size. There was a significant interaction ($P = 0.01$) of cultivar with sampling day for the three smallest leaf-spot categories (Fig. 1). No interactions occurred in 1981. A substantial increase in the number of leaf spots in category A (≤5 cm^2) occurred in all cultivars 8 days after the first signs of disease. After a slight decline during the next sampling period, the number of type A leaf spots increased again 8 days after the last bloom of lesion proliferation (Fig. 1). Lesion expansion and coalescence into larger necrotic areas were not evident until 9–13 days after the first sign of disease. In general, Mississippi Silver and Coronet sustained a greater mean number of leaf spots in all size categories in 1980, whereas Mississippi Silver sustained substantially more leaf spots in 1981 (Table 1). There were no significant differences between White Acre and Pinkeye Purple Hull in numbers of leaf spots by size except in 1980, when White Acre had a greater number of type A leaf spots.

In 1980, number of days had a linear effect on type A leaf spots and quadratic effects on type B, C, and D leaf spots. Regression equations for best fit of the data, where X = number of days, were as follows: type A leaf spots $\sqrt{\text{count} + 0.05} = 2.7 + 0.45X$, $R^2 = 0.68$; type B leaf spots $\sqrt{\text{count} + 0.05} = 1.2 - 0.28X + 0.05X^2$, $R^2 = 0.88$; type C leaf spots $\sqrt{\text{count} + 0.05} = 0.01 + 0.18X + 0.008X^2$, $R^2 = 0.86$; and type D leaf spots $\sqrt{\text{count} + 0.05} = 1.38 - 0.19X + 0.03X^2$, $R^2 = 0.89$.

The R values indicate that 68% of the variation in type A leaf spots was explained by variation in number of days. Variation in number of days and the square of number of days explained 88, 86, and 89% of type B, type C, and type D leaf spots, respectively.

The amount of foliar area per plant at harvest was determined each year for each cultivar by multiplying the mean area of a leaf by the mean number of leaves per plant (Table 2). White Acre had the greatest foliar area because of a significantly greater number of leaves per plant. Mississippi Silver had the next largest foliar area because of its larger leaf size. Coronet and Pinkeye Purple Hull did not differ significantly and were the smallest in terms of foliar area.

The overall disease ratings of the four cultivars of cowpea to BBC were basically similar in field tests in 1980 and 1981 (Fig. 2). Disease progress curves for each cultivar were compared by a Gompertz transformation (2). The purpose of such a transformation was to linearize disease proportions to assist in the interpretation of the sigmoidal curves that are commonly fitted to disease progress data. Rate parameters (k) were calculated (2) and treatment means analyzed statistically. In 1980, Coronet, Mississippi Silver, White Acre, and Pinkeye Purple Hull had k values of 0.091, 0.086, 0.054, and 0.042, respectively (Fig. 2). The k values for Coronet and Mississippi Silver did not differ significantly from one another ($P = 0.01$) but were significantly greater than the grouping of k values for White Acre and Pinkeye Purple Hull. In 1981, there were three groupings of the rate parameters. Mississippi Silver had the highest k value (0.093), significantly greater than 0.071 for Coronet. The k values of 0.034 and 0.029 for White Acre and Pinkeye Purple Hull, respectively, did not differ significantly (Fig. 2).

All bactericidal treatments in 1982 provided significant ($P = 0.01$) control of both foliar and stem canker symptoms compared with no treatments (Table 3). The addition of maneb to copper compounds provided no additional control over that provided by copper compounds alone. Despite excellent control of BBC symptoms, seed harvested from all treatments in 1982 were infested with *X. c. vignicola*. The dilution end point of inoculum of the seed wash water was 10^{-5} for all treatments as determined by a leaf infiltration bioassay (6).

A host range study of bacteriophages P1 and P6 was performed with *Pseudomonas syringae* pv. *syringae* and pv. *tomato, P. avenae, P. solanacearum, X. campestris* pv. *campestris*, pv. *vesicatoria*, pv. *malvacearum*, pv. *pruni*, pv. *phaseoli*, and pv. *vignicola*. The bacteriophages were highly specific and virulent only against *X. c. vignicola*. *X. c. vignicola* strain 80-3 (lysotype A) was sensitive to only P6, and *X. c. vignicola* strain 80-4 (lysotype B) was sensitive to both P1 and P6. In 1983, a copper bactericide again provided significant control of BBC in field plots of Mississippi Silver (Table 3). Periodic sampling of lesions from plants in plots treated with copper yielded only lysotype B in all replicates. Lesions from plants in control plots yielded only lysotype A; however, seed harvested from plants in control plots as well as from plants in treated plots yielded mostly lysotype A. Only the few plants (<15%) with a moderately heavy number of leaf spots yielding lysotype B in treated plots had seed infested with lysotype B.

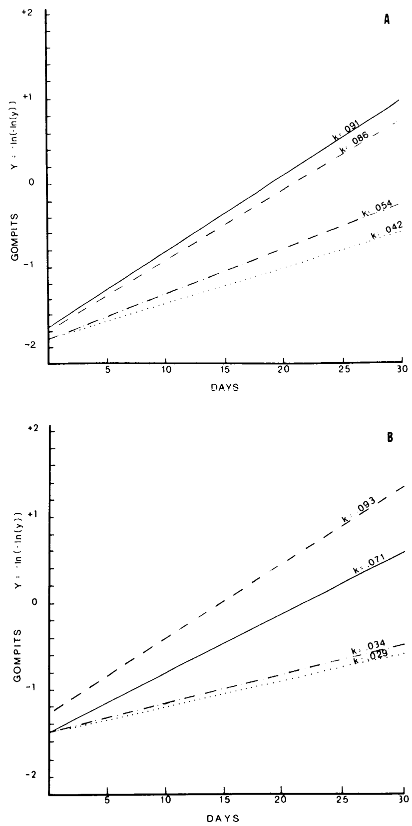

Fig. 2. Regression lines for bacterial blight and canker development in four cowpea cultivars in **(A)** 1980 and **(B)** 1981. Cultivars evaluated were Coronet (——), Mississippi Silver (----), White Acre (-·-·-), and Pinkeye Purple Hull (·····). Regression lines were developed from disease ratings transformed to gompits. k = Gompertz rate parameter.

Table 2. Mean foliar area of four cowpea cultivars at harvest in field tests in 1980 and 1981

Year	Cultivar	Leaf area (cm^2)	Number of leaves/plant	Total foliar area (cm^2)
1980	White Acre	141 b[z]	51 a	7,135 a
	Mississippi Silver	197 a	25 b	4,876 b
	Coronet	164 b	22 b	3,651 c
	Pinkeye Purple Hull	136 b	24 b	3,292 c
1981	White Acre	154 b	57 a	8,724 a
	Mississippi Silver	211 a	27 b	5,768 b
	Coronet	159 b	26 b	4,099 c
	Pinkeye Purple Hull	149 b	28 b	4,205 c

[z] Mean values within a column for an individual year followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 3. Evaluation of bactericides for control of bacterial blight and canker of cowpea and for production of seed free of infestation with *Xanthomonas campestris* pv. *vignicola*

Year	Treatment and rate	Disease severity: Foliar (0–11)	Disease severity: Stem canker (0–5)	Yield (whole pod) (kg/ha)	Dilution end point of seedborne inoculum
1982	Copper hydroxide (2.2 kg/ha)	0.8 a[x]	0 a	5,269 ab	10^{-5}
	Copper ammonium carbonate (7 L/ha)	1.0 a	0 a	5,822 a	10^{-5}
	Copper hydroxide (2.2 kg/ha) + maneb (2.2 kg/ha)	0.5 a	0 a	5,985 a	10^{-5}
	Copper ammonium carbonate (7 L/ha) + maneb (2.2 kg/ha)	0.6 a	0 a	6,116 a	10^{-5}
	Control (no bactericides)	4.6 b	3.5 b	4,261 b	10^{-5}
1983	Copper ammonium carbonate (7 L/ha) + maneb (2.2 kg/ha)	1.7 a	0 a	5,679 a[y]	$10^{-4}/10^{-5}$ [z]
	Control (no bactericides)	4.9 b	2.6 b	4,543 a	10^{-6}

[x] Each value is the mean of four replicates. Means within columns for each year followed by the same letter are not significantly different as determined by an analysis of variance and Duncan's multiple range test ($P = 0.05$).
[y] Yield data in 1983 were influenced by severe deer damage.
[z] Seed harvested in 1983 from treated plots were divided into two groups from either plants without visible symptoms (numerator) from which lysotype A was recovered or from plants with foliar lesions (denominator) from which lysotype B was recovered.

DISCUSSION

BBC poses a threat to the cowpea-processing industry in the southeastern United States. The four major cultivars grown in Georgia represent three distinct pea types: Mississippi Silver is a crowder type, White Acre is a cream pea, and Coronet and Pinkeye Purple Hull are the typical blackeye pea but have a reddish eye preferred for canning. If BBC is of concern, Pinkeye Purple Hull is a superior choice to Coronet for the Pinkeye cultivars. Mississippi Silver was highly susceptible to BBC; consequently, the disease could have its greatest economic impact on crowder pea production.

Cultivar rankings for leaf-spot counts in the early stages of disease were similar to those in the final disease ratings. However, there was no apparent advantage to categorize leaf spots by size nor was there any indication that lesion expansion (as indicated by the size categories) was related to final disease ratings.

Conventional foliar bactericidal treatments, though effective in controlling symptoms and increasing yields, did not prevent seed infestation. Therefore, the value of using bactericides in seed production fields may be questionable. Most seed in treated plots, however, was infested with lysotype A, which was only inoculated onto control plots. The means of dissemination and seed infestation are not known, but passive dissemination by insects is a possibility (9). At blossom, there is extensive insect activity, and possibly, bees and other insects could have carried bacteria of lysotype A from the heavily diseased plants in the check plots into blossoms of symptomless plants in treated plots. The reciprocal transfer of lysotype B to control plots would be less likely because of the fewer and less severe diseased plants with lysotype B. Also, even if lysotype B were transferred into control plots, its detection would be more difficult because there was nearly 100% incidence of lysotype A in control plots. Consequently, the high level of check-plot interference accounting for most of the seedborne infestation in treated plants means that conventional copper bactericides still may be beneficial to reduce, but not eliminate, seedborne inoculum in cowpea.

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