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FULL RESEARCH PAPER

Identification of resistance to Cercospora leaf spot of cowpea

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Abstract Twelve selected cowpea cultivars were screened for resistance to Cercospora leaf spot (CLS) disease caused by *Pseudocercospora cruenta* and *Cercospora apii s. lat.* under artificial epiphytotic conditions in a replicated field trial, with the objective of developing a quantitative measure of disease resistance. CLS incidence, leaf spotting score, lesion density, lesion size, proportion of nodes infected, diseased leaf area, conidia number mg^{-1} and fascicle density were assessed in 12 cowpea genotypes at crop maturity. Proportion of nodes infected and leaf spotting score were best able to quantitatively differentiate between the levels of resistance, and allow the exploitation of quantitative resistance to the disease. Both lesion density and lesion size were important in determining the final leaf spotting score but the former was epidemiologically more important than the latter, indicated by its correlation to most of the CLS symptom measures. There was differential resistance to the *P. cruenta* and *C. apii s. lat.* among the cowpea varieties screened. Among the cowpea lines screened, resistance to *P. cruenta* was more common than resistance to *C. apii s. lat*. Nevertheless, *P. cruenta* was considered the more aggressive and epidemiologically more important than *C. apii s. lat.* on the varieties tested evidenced by the strong correlation of *P. cruenta* incidence with acropetal spread of CLS, intensity of leaf spotting, conidia number mg^{-1} and fascicle density. The highly susceptible varieties namely VRB7, Los Banos Bush Sitao no.1 and CB27 were susceptible to both Cercospora pathogens. The cowpea variety VRB-10 was completely resistant to both pathogens and is a useful source of resistance in CLS breeding programmes.

Keywords *Cercospora canescens* · *Cercospora cruenta* · Disease screening · Quantitative resistance · *Vigna unguiculata*

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Introduction

Cercospora leaf spot (CLS) disease is an important constraint to cowpea (*Vigna unguiculata*) production, particularly in the humid tropics (Schneider et al. 1976). Yield loss attributed to CLS in susceptible cowpea varieties varies between 36% and 42% (Schneider et al. 1976; Fery et al. 1977). CLS disease symptoms are not apparent until the time of flowering (Williams 1975), but can rapidly progress acropetally leading to premature defoliation. Severe infections

can result in lesions developing on pods and stems (Mulder and Holliday 1975a; Williams 1975; Hart 1977; Vakili 1977).

The CLS pathogens, *Pseudocercospora cruenta* (Deighton 1976) and *Cercospora apii s. lat. emend.* (Crous and Braun 2003) (*C. apii s. lat.*; pseudonym *C. canescens*) are widely distributed wherever cowpea is cultivated (Williams 1975; Allen 1983). *Cercospora apii s. lat.* is considered to be a comparatively weaker parasite than *P. cruenta* but has a wider host range in the tropical world (Fery et al. 1976). Leaf spots produced by *C. apii s. lat.* on various hosts are circular, whereas those elicited by *P. cruenta* are mainly indistinct, sometimes angular, and delimited by the venation of the leaf (Mulder and Holliday 1975a, b; Williams 1975). Nevertheless, *C. apii s. lat.* is often found in mixed infections with other Cercosporas, which makes species determination based on symptoms difficult (Braun 1995; Crous and Braun 2003). Where *C. apii s. lat.* is a secondary invader of leaf spots caused by *P. cruenta*, lesion morphology mimics that of leaf spotting caused solely by *P. cruenta*. In Trinidad, although both pathogens of cowpea have been reported (Baker and Dale 1951; Hart 1977), *P. cruenta* was found to be more widespread (Booker 2006).

Resistance to CLS is generally identified by field screening under natural CLS epiphytotics established by either or both susceptible spreader rows and scattering diseased crop debris (Vakili 1977; Williams 1977; Jhooty et al. 1980; Kannaiyan et al. 1987). The artificial culture of Cercosporas is difficult and at the present time a methodology for mass production of virulent conidia on artificial media is not available for Cercosporoid fungi. The majority of researchers opt for the disease nursery system over laboratory or greenhouse testing because abnormal plant reactions to low light and high temperatures can be avoided, and mature plant reactions can be more easily assessed. Under natural epiphytotics, however, it is difficult to determine the differential response of cultivars to a particular pathogen when mixed infections occur. Vakili (1977), however, attempted to identify the specific causal agent(s) of leaf spotting using colour of lesions, length of conidiophores and number of conidiophores per sporangium as indicators, which allowed him to conclude that there was no differential response of cultivars to infection by *Cercospora* species. Vakili (1977), however, noted that the shape of lesions was influenced by cultivar differences in leaf morphology and climatic conditions. Subsequently, Braun (1995) confirmed that *Cercospora* species cannot be identified reliably based on symptoms, as lesion shape, size and colour can vary greatly depending upon host (cultivar). As such, correct identification may require microscopic examination of a representative sample of lesions (Braun 1995).

Numerous sources of resistance to CLS have been identified in cowpea (Vakili 1977; Williams 1977; Jhooty et al. 1980; Kannaiyan et al. 1987; Zhang et al. 1994). The genetic basis of CLS resistance has been characterized as qualitative, determined by either dominant, co-dominant or recessive genes, depending on the cross (Fery et al. 1976; Fery and Dukes 1977; Castro et al. 2003). Genetic analysis of the segregating populations in these studies was, however, based on pooling genotypes into arbitrary categories based on disease reaction scores recorded at maturity or at late pod filling stage. A quantitative screening method capable of discriminating between levels of resistance is required to detect partial resistance. The objectives of the study were: to develop a quantitative measure of resistance to CLS that is precise and capable of discriminating between levels of resistance, and to determine whether there was any evidence of differential resistance to the CLS pathogens, *P. cruenta* and *C. apii s. lat.*

Materials and methods

Location and season

A field experiment was conducted in the wet season, during the period June–August, 2001, at the field station of the University of the West Indies, Valsayn, Trinidad. The soil type at the field station is Fluventic Eutropepts, a flat, deep, alluvial, sandy-loam with free internal drainage (Brown and Bally 1970). The average monthly sunshine hours ranged between 6.6 h and 7.2 h day^{-1}, average daily temperatures varied between a maximum of 31.5–31.9°C to a minimum of 22.8–23.4°C, and the monthly average relative humidity (RH) fluctuated between 70.6% and 75.6%, during the study period. The average monthly precipitation was 194 mm.

Experimentation

Twelve selected pureline varieties of cowpea (Table 1) were evaluated for their resistance to CLS disease in a randomized complete block design with four replications under an artificially induced epiphytotic attained through the use of spreader rows. Each plot as well as the block were surrounded by a spreader row of a susceptible variety (CB27), planted two weeks in advance of the test varieties. The spreader rows were inoculated at the flower initiation stage of the crop with a diseased leaf wash (10 g leaf:1 g H_20; 4.8×10^5 conidia ml^{-1}; 12 ml $plant^{-1}$) between 16.00 h and 18.00 h. The inoculum was applied to the plants with a knapsack sprayer until runoff. In addition, diseased leaf debris was placed at the base of spreader plants. The following morning at 08:00 h a water spray was applied to spreader row plants to maintain high humidity. CLS diseased leaves were obtained from a disease nursery maintained at Field B, University of West Indies (UWI), St. Augustine Campus, Trinidad.

Establishment and after care

The test varieties of cowpea (Table 1) were sown in trays containing peat moss and seedlings transplanted into the field after one week to ensure a uniform stand. The field experiment was established on cambered beds, with each plot consisting of a single test row, 4 m long, with an inter-row spacing of 60 cm and intra-row spacing of 25 cm, guarded on either side by spreader rows. Granular fertilizer Blaukorn for chloride-sensitive crops (12% N, 12% P_2O_5, 17% K_2O, 2% MgO, 6% S, 5% CaO, 0.2% Fe, 0.02% B, 0.01% Zn and traces of Mg, Cu, Mb) was spread along rows at a rate of 200 kg ha^{-1} during molding, 14 days after sowing (DAS). Plots were sprayed every 10 days during the study period / 14–62 DAS, with an insecticide alpha-cypermethrin (Fastac 5 EC, BASF, Colombia) at a rate of 15 g a. i. ha^{-1}. Routine vector control and roughing successfully prevented *Cowpea severe mosaic virus* incidence in plots. Plots were kept weed-free by manual weeding throughout the duration of the study.

Data collection

Flowering dates of individual plants within each plot were recorded. Days to flowering (DTF) was calculated on a plot basis by fitting a straight line through the data points and calculating days to 50% flowering. It was important to make disease assessments on

Table 1 The origin and characteristics of 12 cowpea varieties used in the study

Cowpea variety	Origin	Breeder, Institution	Characteristics
VRB-7	Trinidad & Tobago	P. Umaharan, UWI	Vegetable type; bushy determinate; CPSMV resistant
VRB-2A	Trinidad & Tobago	P. Umaharan, UWI	Vegetable type; bushy determinate; CPSMV resistant
VRB-10	Trinidad & Tobago	P. Umaharan, UWI	Vegetable type; bushy semi-prostrate; CPSMV & CLS resistant
IT-86D-719	Nigeria	IITA, Nigeria	Grain type; bushy determinate; day-neutral; CLS resistant
IT-87D-939–1	Nigeria	IITA, Nigeria	Grain type; bushy determinate; day-neutral; CLS resistant
IT-87D-792	Nigeria	IITA, Nigeria	Grain type; bushy determinate; day-neutral; CLS resistant
IT-87D-400	Nigeria	IITA, Nigeria	Grain type; bushy determinate; day-neutral; CLS resistant
IT-83S-899	Nigeria	IITA, Nigeria	Vegetable type; bushy determinate; short-day
Fond D′or 46–8	Guadeloupe	INRA, Guadeloupe	Grain type; bushy indeterminate; CLS resistant
Los Banos Bush Sitao	Philippines	Univ. of Philippines	Vegetable type; bushy, semi-determinate
Black eye pea	USA	Unknown origin	Grain type; bushy determinate
California Blackeye 27(CB-27)	USA	Patel, PN & Ehlers, JDUCLA, Riverside	Grain type; bushy determinate, early, day-neutral; semi-erect, fusarium wilt resistant (some biotypes), root knot resistant; heat tolerant

UWI = The University of the West Indies; IITA = International Institute for Tropical Agriculture, Nigeria

cowpea lines at the same physiological (days after flowering) rather than chronological age (days after planting) because CLS is not normally apparent in the cowpea crop until the time of flowering (Williams 1975). Disease assessments were therefore made at crop maturity, 25–30 days after flowering (DAF) to avoid the possibility of escapes.

Six bordered plants from each plot were harvested at maximum pod load, 25–30 DAF, placed in polythene bags and transported immediately to the laboratory. Disease incidence and average number of infected nodes per plant were recorded on a plot basis, at the time of destructive harvesting (25–30 DAF). Proportion of infected nodes was determined by dividing the average number of infected nodes per plant by the average number of nodes per plant. Each harvested plant was given a leaf spotting score according to Williams (1977): 0—no symptoms; 1—occasional scattered leaf spots; 2—scattered no more than one per leaflet, on more than half the leaflets; 3—two or three spots per leaflet on few leaves; 4—two or three spots per leaflet on most leaves; 5—many spots on few leaves; 6—many spots on most leaves; 7—withering or abscission of few leaves; 8—withering or abscission of most leaves. The leaf spotting score for each plot was calculated as an average of the six plants. Six leaflets exhibiting the most advanced CLS symptoms were excised from each plant, washed and wrapped in paper toweling and placed in a sealed plastic bag overnight, to allow sporulation. The following morning leaflets were removed and a 4 cm^2 area was sampled from either side of the midrib. The number of lesions and the total lesion area were recorded within each sample area. The lesion area was recorded by tracing the lesions within the area on transparent paper and estimating the area of the cut-outs using a ΔT area meter (Hitachi, Denshi Ltd., Japan). From the data generated, the average number of lesions per unit area and the average lesion size were calculated for each experimental plot.

Lesions were excised using a cork borer from each of the leaflets harvested from a plot, weighed, immersed in sterile distilled water (10 g leaf:1 g H_2O) with a drop of Tween 20 and agitated using a vortex to release the conidia into water. Conidial number ml^{-1} was estimated using a hemacytometer for each experimental plot as described by Tuite (1969).

Pathogen identification was based on six random lesions sampled from each of the six plants per plot. The lesions were excised using a cork borer and mounted on a slide with lactic acid. Slides were heated over a flame to clear the sample, which was subsequently examined under the light microscope for the presence of CLS pathogen(s). Pathogen species determination was made according to keys prepared by Braun (1995) and Braun et al. (1999), and lesions were characterized as single infections of *P. cruenta* or *C. apii s. lat.* or mixed infections of the two. Incidence of *C. apii s. lat.* and *P. cruenta* on representative sample of lesions was used to calculate relative number of leaf spots elicited by each of the pathogens. The centre of the lesion where fruiting is most abundant was located, and the number of *P. cruenta* and/or *C. apii s. lat.* conidiophore fascicles was counted within the field of view using a 10× objective and converted to number of fascicles per unit area.

Data analysis

Results were analyzed using the statistical software package Minitab for Windows version 14 (State College, PA, USA). To normalize data an arcsin transformation was performed on the proportion of CLS—infected plants at crop maturity, proportion of CLS—infected nodes, proportion of CLS diseased leaf area, incidence of *P. cruenta* and/or *C. apii s. lat.* on lesions, and a natural log+1 transformation was carried out on lesion density and conidia count mg^{-1}. Analysis of variance (general linear model) and *F*-tests were performed to determine cultivar differences for the various measures of resistance to CLS. Mean separation was based on Tukey's pair-wise comparison test. Index of differentiation for each disease response was calculated using the following formula: coefficient of variation (CV) between genotypes divided by CV within genotypes. Phenotypic and genetic correlation analysis (Pearson's product moment correlation) was carried out to ascertain the degree of linear relationship between the all the measures of resistance, across genotypes. Phenotypic and genetic correlation involved correlation of replicate data values and genotypic mean values, respectively, for all disease measures. Multiple regression analysis was done to determine the relative contributions of lesion density and lesion size on diseased leaf area and leaf spotting score.

Results

The cowpea varieties screened for resistance to CLS in this study represent diverse origins (Nigeria, the Philippines, the Caribbean and the continental United States), and encompass both vegetable (Cultigroup Sequipedalis) and grain (Cultigroup Unguiculata) types (Table 1). There was significant ($P < 0.05$) variation in the time to 50% flowering (39–53 DAF) among the varieties studied. Plant physiological age is important in determining the onset of CLS and therefore assessments on cowpea lines were made at 25–30 DAF.

Measures of resistance to CLS

There was significant ($P < 0.001$) variation (0–1) in the proportion of plants that became infected at crop maturity (Table 2). Five varieties, IT-87D-400, VRB-7, Bush Sitao, black eye pea and CB27 showed 100% or near 100% infection under the epiphytotic conditions created. IT86D-719 and VRB 2A had 60% and 78% of plants infected, respectively, while another four varieties (Fond D′or, IT87D-792, IT87D-939–1 and IT83S-899) had <25% of plants infected. VRB-10 was the only variety that was completely free of symptoms.

Of the five varieties that showed 100% infection, the infection had spread to leaves of all the nodes in four of the varieties with the exception of IT-87D-400 (Table 2). In the latter, infection had moved to only 40% of the nodes. There was a general correlation between the proportion of plants infected and the proportion of nodes infected in the remainder of the varieties with the exception of IT-86D-719, where although 60% of plants were infected, only 23% of nodes, on average, were infected per plant.

There were significant ($P < 0.001$) differences in the leaf spotting score and density of leaf spotting among the varieties tested (Table 2). The leaf spotting score closely reflected the proportion of nodes infected ($r = 0.99$). The lesion density (number cm^{-2}) showed a trend similar to the leaf spotting score with the exception of VRB-2A, which had a

Table 2 Cercospora leaf spot disease incidence, intensity of leaf spotting and relative incidences of *C. apii s. lat.* and *P. cruenta* in 12 lines of cowpea

Genotypes	DTF[a] 50%		Proportion of				Leaf spotting				*C. apii*		*P. cruenta*	
			Plants infected		Nodes infected		Score[b]		no. cm^{-2}		no. cm^{-2}		no. cm^{-2}	
VRB 10	40.5	ab[c]	0.00	c	0.00	c	0.00	c	0.00	d	0.00	c	0.00	c
Fond D′or	44.9	bc	0.03	c	0.01	c	0.08	c	0.22	d	0.22	bc	0.00	c
IT87D-792	53.2	e	0.05	c	0.01	c	0.08	c	0.16	d	0.16	bc	0.16	c
IT87D-939-1	49.5	cd	0.11	c	0.19	bc	1.92	b	0.56	cd	0.43	bc	0.47	c
IT83S-899	46.8	cd	0.25	c	0.22	bc	2.5	b	1.50	bc	0.96	bc	1.40	bc
IT86D-719	46.1	c	0.60	b	0.23	bc	1.00	bc	1.00	bc	1.00	bc	0.00	c
IT87D-400	42.8	b	1.00	a	0.41	b	3.08	b	3.09	ab	3.09	ab	0.00	c
VRB 2A	40.4	ab	0.78	ab	0.74	a	5.08	a	0.94	cbd	0.40	b	0.66	c
VRB 7	41.6	ab	0.97	a	0.89	a	5.58	a	5.31	a	4.58	a	5.30	ab
Bush Sitao	42.8	b	0.99	a	0.98	a	6.08	a	4.47	a	2.46	abc	4.09	ab
Black eye pea	39.4	a	0.99	a	1.00	a	6.5	a	5.66	a	1.17	bc	5.62	a
CB27	39.5	a	1.00	a	0.99	a	6.42	a	3.44	a	1.72	bc	1.92	bc
Significance ($P<$)	0.0001		0.0001		0.0001		0.0001		0.0001		0.001		0.0001	
SE	0.59		0.080		0.058		0.385		0.658		0.665		0.579	
CV (within genotypes)	0.027		0.284		0.247		0.241		0.600		0.987		0.709	
Index of Diff.	3.65		2.74		3.57		3.40		1.60		1.05		1.85	

[a] DTF = days to 50% flowering

[b] The leaf spotting score based on Williams (1977)

[c] Means followed by different letter are significantly different according to Tukey pair-wise comparisons ($\alpha \leq 0.05$) or ($\alpha \leq 0.10$) for means followed by italicized letter

smaller lesion density compared to the leaf spotting score. Varieties VRB-10, Fond D′or, IT-87D-792 and IT-87D-939–1 had <1 leaf spot cm^{-2}, IT-86D-719, IT-83S-899 and VRB-2A had 1–2 leaf spots cm^{-2}, while the remaining five varieties had >3 leaf spots cm^{-2} (Table 2).

There was evidence of differential susceptibility of cowpea varieties to *P. cruenta* and *C. apii* (Table 2). Only VRB-10 showed complete resistance to both pathogens. Likewise, VRB-7 and Bush Sitao were highly susceptible to both pathogens. On the contrary, IT87D-400 and IT-86 D-719, which were resistant to *P. cruenta*, were highly and moderately susceptible to *C. apii*, respectively. Similarly, blackeye pea was highly susceptible to *P. cruenta*, but only moderately so to *C. apii*. In varieties susceptible to both pathogens, mixed infections were quite common. In varieties IT87D-400 and IT-86 D-719 susceptible to *C. apii* but not *P. cruenta*, the proportion of nodes infected was lower (Table 2).

Of the various measurements of resistance to CLS disease outlined in Table 2, proportion of nodes infected and the whole plant leaf spotting score had the lowest CV and consequently the highest index of differentiation. These measurements are hence best suited to differentiate varieties based on their levels of susceptibility. The high CV associated with leaf spot density measurements may be due to a large sampling error, particularly since leaf spotting densities may not be uniform from leaf to leaf.

There were significant differences ($P < 0.01$) in lesion size among the varieties tested varying between 0 (VRB-10) and 70.2 mm^2 (VRB-2A) (Table 3). However due to the relatively large CV and relatively low index of differentiation, it was not possible to differentiate levels of intermediate resistance.

There were significant differences ($P < 0.0001$) in diseased leaf area among the cowpea varieties tested (Table 3). Multiple regression analysis showed that lesion density and lesion size together were able to explain 76% of the variation in diseased leaf area and 90% of the variation in whole plant leaf spotting score. Hence whole plant leaf spotting score provided a better synthesis of the combined effect of lesion density and lesion size rather than diseased leaf area.

Table 3 The ability of *Cercospora* spp. to proliferate (conidial and fascicle number) and cause leaf symptoms (lesion density, size and diseased leaf area) in 12 lines of cowpea

Genotypes	Cercospora leaf lesion				Proportion Diseased		Fascicle no.		Conidia	
	no. cm^{-2}		av. size mm^2		Leaf Area		mm^{-2}		per mg leaf	
VRB 10	0.00	*d*	0.0	b	0.00	bcd	0.0	cd	0	cd
Fond D′or	0.22	*d*	4.3	b	0.04	bcd	3.0	cd	0	cd
IT87D-792	0.16	*d*	2.0	b	0.01	bcd	8.5	cd	36	bcd
IT87D-939-1	0.56	*cd*	20.8	ab	0.11	bcd	58.4	abc	264	abc
IT83S-899	1.50	*bc*	32.2	a	0.42	abc	70.3	abc	793	ab
IT86D-719	1.00	*bc*	26.2	ab	0.33	bcd	24.6	cd	28	bcd
IT87D-400	3.09	*ab*	18.2	ab	0.37	bcd	28.7	bcd	72	abc
VRB 2A	0.94	*cbd*	70.3	a	0.39	bcd	86.7	abc	1060	abc
VRB 7	5.31	*a*	10.8	ab	0.50	ab	92.9	ab	135	abc
Bush Sitao	4.47	*a*	10.8	ab	0.52	ab	95.4	ab	307	abc
Black eye pea	5.66	*a*	7.9	ab	0.42	abc	94.8	ab	612	ab
CB27	3.44	*a*	21.2	ab	0.73	a	67.2	abc	7950	ab
Significance (*P*<)	0.0001		0.01		0.0001		0.0001		0.0001	
SE	0.66		11.35		0.088		11.7		984	
CV (within genotypes)	0.600		1.21		0.557		0.445		2.09	
Index of Diff.	1.6		0.8		1.3		1.6		1.1	

Means followed by different letter are significantly different according to Tukey pair-wise comparisons ($\alpha \leq 0.05$) or ($\alpha \leq 0.10$) for means followed by italicized letter

Furthermore, leaf spotting score had a much larger index of differentiation than diseased leaf area (Tables 2 and 3).

There were significant ($P < 0.0001$) differences in both fascicle density and conidial number mg^{-1} between the varieties tested (Table 3). However, the CV was particularly high for conidial number mg^{-1} indicating that environment plays a larger role than varietal differences in conidial number mg^{-1}. Fascicle density, on the other hand, was found to be a much better measure of pathogen proliferation in the tissue, and correlated with lesion density, number of plants infected, proportion of nodes infected, leaf spotting score and diseased leaf area, but not with lesion size (Table 4).

Inter-relationships between the resistance measures

Table 4 provides the phenotypic and genotypic correlation coefficients between various measurements on their appropriate transformed scales. As expected, the genotypic correlation coefficients were higher than phenotypic correlation coefficients. The difference was particularly large for conidial production, where environmental factors other than genotypic factors would have also had a large influence on conidial production.

DTF showed small but negative trends with all disease measurements, but the genotypic correlation coefficients were significant ($P < 0.05$) only for disease incidence, leaf spotting score and proportion of nodes infected. This indicated that the later flowering varieties tended to have a somewhat smaller CLS disease incidence and proportion of nodes infected and leaf spotting score (Table 4).

The genotypic correlation (Table 4) between the density of *P. cruenta* leaf spotting and fascicle density, conidial production, disease incidence and proportion of infected nodes or leaf spotting score was large and significant ($P < 0.01$ or $P < 0.001$). In comparison, the genotypic correlation between densities of *C. apii* leaf spotting and fascicle density or conidial production was not significant. Although the genotypic correlation between *C. apii* spotting and proportion of nodes infected or leaf spotting score was significant, it was much lower in magnitude than those for *P. cruenta* spotting (Table 4). These results

Table 4 Pearson's product moment correlation between various disease measures (transformed data) obtained for 12 varieties of cowpea (a) phenotypic correlation (shaded) and (b) genotypic correlation (unshaded).

	Lesion no.	Lesion size	[a]DLA	Fascicle no.	Conidia no.	Incidence	Proportion infected nodes	CLS Score	C. apii leaf spots	P. cruenta leaf sports	[b]DTF 50%
Lesion no.	1	−0.05	0.70***	0.70***	0.63***	0.78***	0.74***	0.79***	0.73***	0.77***	−0.51***
Lesion size	0.01	1	0.36**	0.29**	0.31**	0.29**	0.23	0.27	−0.11	−0.14	−0.12
DLA	0.78***	0.36	1	0.64***	0.68***	0.72***	0.63***	0.68***	0.44***	0.40***	−0.47***
Fascicle no.	0.79***	0.4	0.66**	1	0.76***	0.57***	0.67***	0.73***	0.46***	0.64***	−0.34**
Conidia no.	0.74***	0.53	0.83***	0.90***	1	0.57***	0.55***	0.64***	0.32**	0.47***	−0.27
Incidence	0.89***	0.21	0.78***	0.66**	0.66**	1	0.80***	0.82***	0.53***	0.49***	−0.62***
Proportion infected nodes	0.87***	0.14	0.76***	0.83***	0.76***	0.86***	1	0.95***	0.35**	0.65***	−0.62***
CLS Score	0.90***	0.29	0.81***	0.90***	0.86***	0.88***	0.99***	1	0.41***	0.66***	−0.60***
C. apii leaf spots	0.79***	−0.09	0.57	0.50	0.4	0.74**	0.59**	0.58**	1	0.57***	−0.28
P. cruenta leaf spots	0.84***	−0.18	0.49	0.79***	0.57**	0.62**	0.82***	0.78***	0.59**	1	−0.43***
DTF	−0.54	−0.18	−0.55	−0.42	−0.38	−0.65**	−0.66**	−0.64**	−0.34	−0.45	1

[a] DLA = disease leaf area

[b] DTF = days to 50% flowering

* significance at $P < 0.05$; ** significance at $P < 0.01$; *** significance at $P < 0.001$

suggest that although both pathogens are responsible for CLS incidence, the susceptibility to *P. cruenta* is associated with greater severity as indicated by the greater correlation to fascicle and conidial density, proportion of nodes infected per plant and leaf spotting score.

There was no significant ($P > 0.05$) genotypic correlation between lesion density and lesion size ($r = 0.01$). While lesion density was highly correlated to disease measures such as incidence, proportion of infected nodes, diseased leaf area and leaf spotting score (Table 4), the genotypic correlations of lesion size to the disease measures were not significant ($P > 0.05$). Nevertheless, lesion size and lesion density together were better able to explain the variation in diseased leaf area or leaf spotting score than lesion density alone. This indicates that lesion size is a component of CLS disease severity, although to a lesser extent than lesion density.

Discussion

The sources of resistance to CLS disease in *V. unguiculata* identified in this study confirm identified sources of resistance from a number of previous studies (Vakili 1977; Williams 1977; Kannaiyan et al. 1987; Zhang et al. 1994; Lin et al. 1995). Nevertheless, many varieties of cowpea identified as resistant in previous studies were shown in this study to have various levels of susceptibility based on a number of CLS disease measurements. Sud and Singh (1984) found that weather conditions influenced the development of CLS epiphytotics in Urdbean, while Fery and Dukes (1977) reported a strong environmental influence on expression of CLS resistance from season to season for populations generated from a resistant x susceptible cross.

The low levels of disease incidence observed in some cowpea varieties under the uniform disease pressure established in this study showed that these varieties possess useful levels of resistance. CLS disease incidence and leaf spotting score (a measure of disease severity) were highly correlated ($r = 0.88$) in this study. Multiple regression analysis showed that both lesion density and lesion size were important in explaining 90% of the variation in the CLS leaf spotting score (CLS leaf spotting score = 1.142 lesion density + 0.056 lesion size − 0.37). This observation along with the finding that lesion density and lesion size are not correlated suggests that resistance to CLS disease may be governed by two independent resistance mechanisms, one governing the lesion density and another controlling the ability of the pathogen to elicit large lesions. The strong correlation of lesion density to disease incidence, proportion of nodes infected, leaf spotting score, as well as fascicle density and conidial number mg^{-1} suggest that lesion density may be epidemiologically the more important resistant component of the two.

The moderate but significant phenotypic correlation but no genotypic correlation between lesion size and diseased leaf area, fascicle density or conidial number mg^{-1} suggests that lesion expansion may be affected by environmental factors rather than genetic determinants. For instance, *Cercospora* species have been recorded to produce a phytotoxin, cercosporin—a light activated perylenequinone (Daub et al. 2005). The host response to the light activated toxin may therefore partly account for the variation in lesion sizes observed in this study. Hence, while larger lesion sizes may mirror the inherent ability of the pathogen to multiply and spread in the host tissue it may also be a reflection of the sensitivity of the host tissue to the toxin produced, which itself is environmentally modulated.

A number of researchers (Fery et al. 1976; Mishra et al. 1988; Thakur et al. 2002; Castro et al. 2003) investigating the mode of inheritance to CLS resistance in *Vigna.* spp. concluded that a single major gene was responsible for CLS resistance. Allen (1983) argued that the predominance of single gene resistance sources identified for CLS may be an artifact of grouping various symptoms into two arbitrary groups, and suggested that an oligogenic or polygenic model may more accurately reflect the inheritance of CLS resistance, but would require quantitative measures of resistance. The present study, for the first time, has identified quantitative assessment of the levels of resistance to CLS disease.

The current study demonstrated conclusively that cowpea varieties screened exhibited a differential response to infection by *P. cruenta* and *C. apii s. lat.* For instance, IT87D-400 and IT-86 D-719, which were highly resistant to *P. cruenta*, were highly and moderately susceptible to *C. apii*, respectively. Similarly, black eye pea which was highly susceptible to *P. cruenta* was only moderately so to *C. apii.*

Only VRB-10 showed complete resistance to both pathogens. The present study therefore confirms that Cercosporoid pathogens, *P. cruenta* and *C. apii*, require microscopic examination of a representative sample of lesions from individual plants, as suggested by Braun (1995).

Pseudocercospora cruenta represents a distinct pathogen species that is host-specific (*Vigna* spp. *Phaseolus* spp.), while *C. apii s. lat.* is a compound species infecting numerous hosts of different families (Crous and Braun 2003). *Cercospora apii*-like hyphomycetes form a morphologically, rather uniform, complicated assemblage of taxa in which the process of speciation is, perhaps, incomplete (Crous and Braun 2003). In comparison, molecular studies consign species placed in *Pseudocercospora* as a distinct clade within the monophyletic Cercosporoid genera (Crous and Braun 2003). In this study, while four cowpea varieties were highly resistant to *P. cruenta* (VRB-10. Fond D′or, IT87D-400 and IT86D-719), only one (VRB-10) was found to be highly resistant to *C. apii s. lat*.

Correlation analysis showed that the level of susceptibility to *P. cruenta* was strongly associated with fascicle and conidial density, proportion of nodes infected per plant and leaf spotting score, while the level of susceptibility to *C. apii s. lat*. was only moderately associated or not associated at all with the above measurements. This suggests that *P. cruenta* is the more aggressive of the two pathogens and therefore epidemiologically the most important, and this is in agreement with Williams (1975) who reported *C. apii s. lat.* to be a weaker pathogen of cowpea. More recently, Crous and Braun (2003) reported that *C. apii s. lat.* forms leaf spots on weakened or senescing host plants and is often a secondary invader of leaf spots.

The results presented in this paper suggest that breeding for resistance should mainly target *P. cruenta*, which is the more aggressive pathogen of the two. It is not only capable of creating significant leaf spotting but is also able to move to younger non-senescing leaves, and hence can significantly reduce the photosynthetic capacity of the plant. *Cercospora apii*, is less important as it is a secondary pathogen or a pathogen of the lower senescing leaves. Proportion of nodes infected and leaf spotting score were best able to quantitatively differentiate between the levels of resistance, and allow the exploitation of quantitative resistance to the disease. This study, for the first time, identified complete resistance to both pathogens in variety VRB-10, which should be used as a source of resistance to Cercosporoid diseases in cowpea breeding programmes.

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References

Allen, C. J. (1983). *The pathology of tropical food legumes*. Chichester, UK: John Wiley & Sons.

Baker, R. E. D., & Dale, W. T. (1951). *Fungi of Trinidad and Tobago*. Kew, Surrey, UK: The Commonwealth Mycological Institute, Mycological Papers 33.

Booker, H. M. (2006). *Studies on resistance to Cercospora leaf spot (CLS) disease and the impact of CLS and Cowpea severe mosaic virus (CPSMV) diseases on productivity of Vigna unguiculata (L.) Walp. in Trinidad.* PhD dissertation, The University of the West Indies, St. Augustine, Trinidad and Tobago.

Braun, U. (1995). A monograph of *Cercosporella*, *Ramularia* and allied genera (phytopathogenic hyphomycetes) vol. 1 IHW-Verlag, Eching bei München, Germany.

Braun, U., Mouchacca, J., & McKenzie, E. H. C. (1999). Cercosporoid hyphomycetes from new Caledonia and some other South Pacific Islands. *New Zealand Journal of Botany*, *37*, 297–327.

Brown, C. B., & Bally, G. S. (1970). *Land capability survey of Trinidad and Tobago: Central Range, No. 4*. Port of Spain, Trinidad: Government Printery.

Castro, N. R., Menezes, G. C., & Coelho, R. S. B. (2003). Inheritance of the genetic resistance of caupi to Cercosporiose. *Fitopathology*, *28*(5), 552–554.

Crous, P. W., & Braun, U. (2003). *Mycospaerella and its anamorphs: 1. Names published in Cercospora and Passalora*. Utrecht, The Netherlands: Centraalbureau voor Schimmelcultures (CBS).

Daub, M. E., Herrero, S., & Kuang-Ren, C. (2005). Photoactivated perylenequinone toxins in fungal pathogenesis of plants. Edited by R.C. Staples. *FEMS Microbiology Letters*, *252*, 197–206.

Deighton, F. C. (1976). Studies on *Cercospora* and allied genera.VI. *Psuedocercospora* Speg., *Pantospora* Cif. and *Cercoseptoria* Petr. *Mycological Papers*, *140*, 1–168.

Fery, R. L., Dukes, P. D., & Cuthbert, F. P. Jr. (1976). The inheritance of Cercospora leaf spot resistance in southernpea (*Vigna unguiculata* (L.) Walp.). *Journal of the American Society for Horticultural Sciences*, *101*(2), 148–149.

Fery, R. L., & Dukes, P. D. (1977). An assessment of two genes for Cercospora leaf spot resistance in the

southernpea (*Vigna unguiculata* (L.) Walp.). *Journal of the American Society for Horticultural Sciences*, *12*(5), 454–456.

Fery, R. L., Dukes, P. D., & Cuthbert , F. P. Jr. (1977). Yield loss of southern pea (*Vigna unguiculata*) caused by Cercospora leaf spot. *Plant Disease Reporter*, *61*(9), 741–743.

Hart, R.O. (1977). *Etiology of Cercospora cruenta Sacc. on Vigna unguiculata (L.) Walp.* M.Sc. dissertation, The University of Guyana, Turkeyen, E.C.D. Guyana.

Jhooty, J. S., Sokhi, S. S., Sohi, H. S., Bains, S. S., Rewal, H. S., & Mushi, G. D. (1980). The reaction of cowpea germplasm to different diseases. *Crop Improvement*, *7*(1), 25–31.

Kannaiyan, J., Greenberg, D. C., Haciwa, H. C., & Mbewe, M. N. (1987). Screening cowpea for resistance to major diseases in Zambia. *Tropical Grain Legume Bulletin*, *34*, 23–26.

Lin, M. C., Chen, H. P., Wang, Y. F., & Zhang, W. Z. (1995). Evaluation of cowpea varieties resistant to cowpea leaf mould (*Cercospora cruenta* Sacc.). *Crop Genetic Resources*, *4*, 36–37.

Mulder, J. L., & Holliday, P. (1975a). *Cercospora canescens*. CMI descriptions of pathogenic fungi and bacteria No. 462. Kew, Surrey, UK: The Commonwealth Mycological Institute.

Mulder, J. L., & Holliday, P. (1975b). *Cercospora cruenta.* CMI descriptions of pathogenic fungi and bacteria No. 463. Kew, Surrey, UK: The Commonwealth Mycological Institute.

Mishra, S. P., Asthana, A. N., & Yadav, L. (1988). Inheritance of Cercospora leaf spot resistance in mung bean, *Vigna radiata* (L.) Wilczek. *Plant Breeding*, *100*, 228–229.

Schneider, R. W., Williams, R. J., & Sinclair, J. B. (1976). Cercospora leaf spot of cowpea: models for estimating yield loss. *Phytopathology*, *66*, 384–388.

Sud, V. K., & Singh B. M. (1984). Effect of environmental factors on the development of leaf spot (*Cercospora canescens*) in urdbean. *Indian Phytopathology*, *37*(3), 511–515.

Thakur, R. P., Patel, P. N., & Verma, J. P. (2002). Genetical relationships between reactions to bacterial leaf spot, yellow mosaic and *Cercospora* leaf spot in mungbean (*Vigna radiata*). *Euphytica*, *26*, 765–774.

Tuite, J. (1969). *Plant pathological methods: fungi and bacteria*. Minneapolis, USA: Burges Publishing Company.

Vakili, N. G. (1977). Field screening of cowpeas for Cercospora leaf spot resistance. *Tropical Agriculture (Trinidad)*, *54*(1), 70–76.

Williams, R. J. (1975). Diseases of cowpea (*Vigna unguiculata* (L.) Walp.) in Nigeria. *Proceedings National Academy of Sciences*, *21*(3), 253–267.

Williams, R. J. (1977). Identification of multiple disease resistance in cowpea. *Tropical Agriculture (Trinidad)*, *54*(1), 53–59.

Zhang, W. Z., Wang, Y. F., & Lin, M. C. (1994). Genetic distance estimation and cluster analysis of some yard long bean resources. *Acta Horticulturae Sinica*, *21*(2), 180–184.