Pictorial Assessment Key to Determine Fungicide Concentrations That Control Anthracnose Development on Cucumber Cultivars with Varying Resistance Levels

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ABSTRACT

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A pictorial assessment key was developed using photographs of cucumber leaves naturally infected by *Colletotrichum lagenarium*. The rating scale (1–8) was divided into ranges of 0–1, 1–3, 3–6, 6–12, 12–25, 25–50, 50–75, and 75–100% diseased tissue. The assessment key was used to evaluate anthracnose development at two locations on two cucumber cultivars with genes that condition differing levels of resistance. These cultivars received four rates of chlorothalonil weekly. Higher rates of chlorothalonil application and inoculation of the central four to six plants compared with whole-plot inoculation resulted in less anthracnose development, measured as area under the disease progress curve or final disease severity. The greater resistance to anthracnose in the cultivar Calico compared with that in Calypso was equal to about 0.4 kg of chlorothalonil applied weekly. This fungicide equivalent was not altered by environment but was affected by inoculum distribution and the disease variable used to calculate the fungicide equivalent.

Additional key words: *Cucumis sativus*

Anthracnose of cucurbits caused by *Colletotrichum lagenarium* (Pass.) Ell. & Halst. is a major leaf spot disease of cucumber (*Cucumis sativus* L.) in the southeastern United States. Resistant cultivars have been developed from Plant Introduction (PI) 197087, which has a very high level of polygenic resistance to *C. lagenarium* (9). The level of polygenic resistance of commercial cucumber cultivars is variable. The resistance of cultivars reduces the rate of disease development. When conditions are favorable for anthracnose, severe epidemics can develop even in the most resistant cultivars.

Frequent applications of fungicides are used to reduce the rate of development of many foliar diseases. Since many of these fungicides reduce the percentage of spores that establish lesions (10), a reduced rate of fungicide application may increase the number of spores that establish these lesions (1). The combination of increasing levels of polygenic, rate-reducing resistance with decreasing fungicide rates could provide adequate plant protection and meet reduced-fungicide requirements (2). The most efficient use of fungicide would be to apply a reduced rate according to a disease forecast (3).

Methods developed to estimate disease severity reflect different research approaches that have involved such areas as disease-loss appraisal, epidemiology, and disease resistance (7). Assessment keys are a useful tool in evaluating disease, as indicated by the large number that have been developed (8). None of these assessment keys are suitable for evaluation of anthracnose on cucumbers. The objectives of this study were to develop a disease severity assessment key (DAK) for anthracnose on cucumbers and to determine rates of weekly chlorothalonil application that allow for anthracnose development similar to pickling cucumber cultivars that possess genes that condition different levels of rate-reducing resistance.

MATERIALS AND METHODS

Leaves of the cucumber cultivar Calypso naturally infected with *C.*

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Table 1. Averages and analysis of variance of fungicide equivalents from tests in two environments comparing the influence of two inoculum distributions and two disease variables used to calculate the fungicide equivalents

	AUDPC[a]		Final disease[b]	
Environment	**Central[c]**	**Whole plot**	**Central**	**Whole plot**
Clinton	0.46	0.33	0.40	0.03
Castle Hayne	0.97	0.36	0.33	0.10

Source of variation[d]	**Degrees of freedom**	**Sum of squares**	**Mean squares**	***F*[e]**
Inoculum distribution	1	0.875	0.875	8.91*
Inoculum distribution × replicate	6	0.589	0.098	...
Environment (location)	1	0.142	0.142	1.44 NS
Environment × replicate	6	0.591	0.099	...
Disease variable (AUDPC or final disease)	1	0.784	0.784	6.22*
Disease variable × replicate	6	0.756	0.126	...
Error	10	0.812	0.081	...
Corrected total	31	4.548	...	...

[a] Area under the disease progress curve was calculated until the first plot in each environment reached 50% diseased tissue, which occurred 56 and 60 days after planting at Castle Hayne and Clinton, respectively. The area was calculated by averaging two consecutive disease evaluations, multiplying by the number of days between observations, and summing these values.

[b] The final disease evaluation was made 60 and 63 days after planting at Castle Hayne and Clinton, respectively.

[c] Inoculum was placed on the central four to six plants or spread on the whole plot, resulting in 0.01 and 0.1% diseased tissue, respectively.

[d] The variance of data from the two environments was significantly different at $P = 0.05$, and the data from both locations was combined in the analysis.

[e] * = Statistical significance ($P = 0.05$) and NS = not significant.

lagenarium were removed from fields and photographed. The leaves were projected onto a Tektronix 4956 digitized board attached to a Tektronix 4051 Graphic System (Tektronix Inc., Beaverton, OR) and the lesion and leaf areas were measured. The rating scale (1–8) used had increasing ranges of diseased tissue: 1 = 0–1, 2 = 1–3, 3 = 3–6, 4 = 6–12, 5 = 12–25, 6 = 25–50, 7 = 50–75, and 8 = 75–100% diseased tissue. The scale of the DAK was chosen to separate disease levels according to the ability of the eye to act as a photocell (5). The upper half of the scale (>50%) was chosen not to be a mirror image of the lower half because leaves with high levels of disease (>75%) rapidly become totally necrotic. Photographs of three leaves representing the range of lesion numbers were included in each scale level (Fig. 1).

Field plots were established at the Horticultural Crops Research Stations at Clinton and Castle Hayne, NC, in May 1982. The experimental design was a split block in which treatments were arranged in a randomized complete block with four replicates. Cultivars were the whole blocks and the fungicide concentration by inoculum placement treatments were the subblocks. Individual plots were five rows wide and 4.6 m long with one unplanted row between plots and 1.5-m alleyways at each end. Rows were spaced at 1.0 and 1.1 m at Castle Hayne and Clinton, respectively. Moderately resistant Calypso and more resistant Calico were planted on raised beds (0.5 m wide) and thinned after the first two true leaves appeared to a density of 49,000–59,000 plants per hectare. Recommended herbicides, insecticides, and fertilizers were applied as needed (6). Overhead sprinkler irrigation was applied as needed to provide about 2.5 cm of water per week.

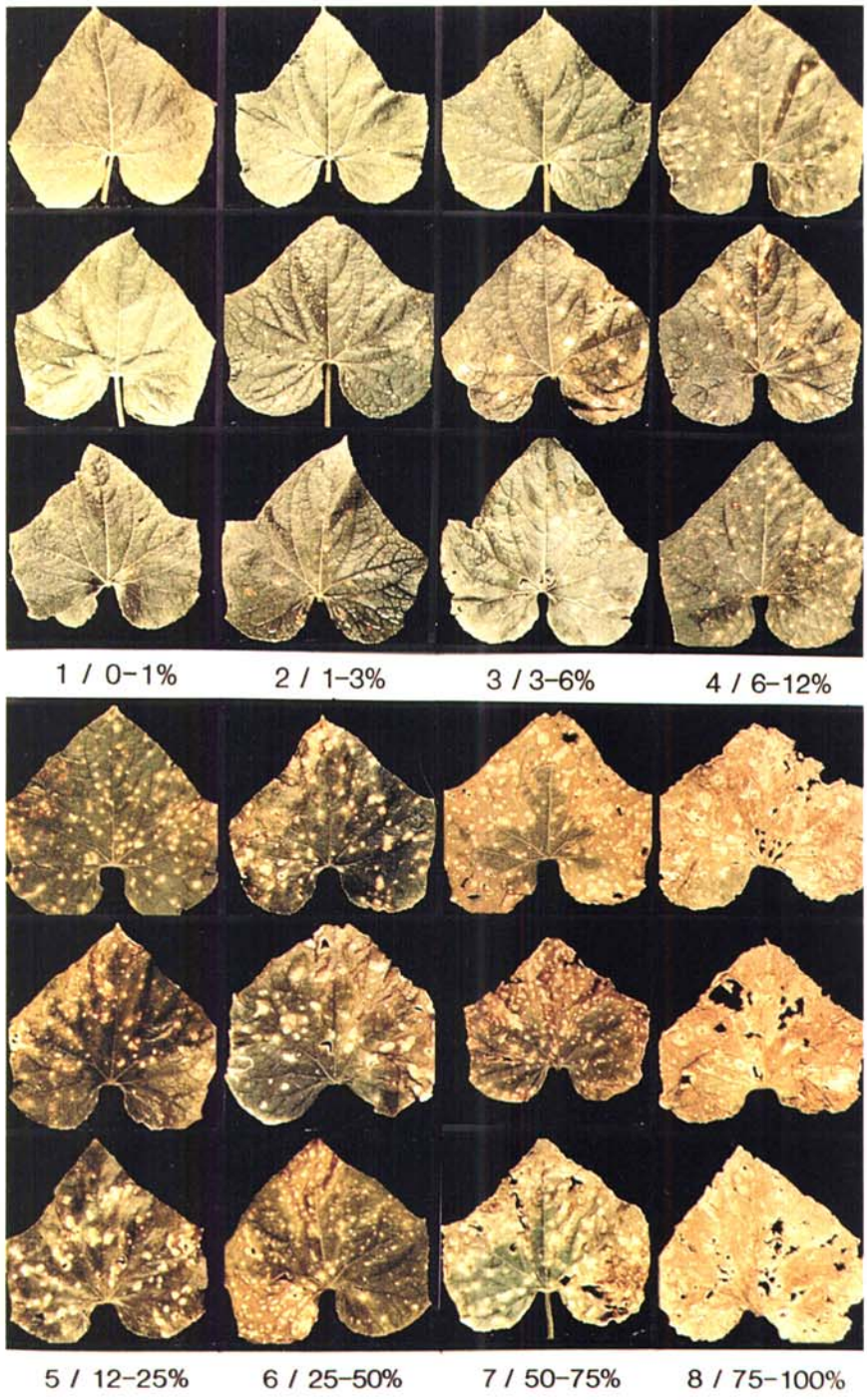

Fig. 1. Disease assessment key of *Colletotrichum lagenarium* on leaves of *Cucumis sativus* cv. Calypso. The key is divided into a rating scale of 1–8, where 1 = 0–1, 2 = 1–3, 3 = 3–6, 4 = 6–12, 5 = 12–25, 6 = 25–50, 7 = 50–75, and 8 = 75–100% diseased tissue.

Conidia of *C. lagenarium* race 1 (ATCC 52609) were removed from 4- to 10-day-old mass cultures growing on green bean agar (4) by gently swirling 0.1 ml of sterile distilled water in the plate. The conidial suspension was calibrated to 25,000/ml with a hemacytometer. Walking at 4.8 km/hr (3 mph), an operator carrying a backpack sprayer atomized the conidial suspensions onto plants at 137.9 kPa (20 psi). Inoculum (15 ml/plot) was applied between 1800 and 2100 hours at the two- to four-leaf stage to either the central four to six plants or to the whole plot. This resulted in 0.01 and 0.1% diseased tissue in the central and whole-plot-inoculated plots, respectively. The two control plots were not inoculated.

After inoculation, chlorothalonil (Bravo 500, 0.5 kg/L) was applied on a 7-day schedule at rates of 0, 0.29, 0.59, 1.17, and 2.34 kg/ha. A hand-held boom applied the fungicide in 935 L/ha (100 gal/acre) at 689 kPa (100 psi) through Tee Jet hollow-cone nozzles (D4 core, 45-disc, Spraying Systems Co., Wheaton, IL). Chlorothalonil was applied at 2.34 kg/ha to one of the uninoculated plots.

Two weeks after inoculation, the first disease evaluation was made by estimating percent diseased tissue of 10 randomly selected leaves per row and 50 leaves per plot, using the DAK and calculating the average. Disease was subsequently evaluated twice a week using the DAK to rate 20–30 randomly selected leaves per plot. The area under the disease progress curve (AUDPC) was calculated by averaging two consecutive disease evaluations, multiplying the average by the number of days between the observations, and summing these values. The AUDPC was calculated until one of the plots at each location reached 50% diseased tissue, which occurred 56 and 60 days after planting at Castle Hayne and Clinton, respectively. Final disease evaluations were made 60 and 63 days after planting at Castle Hayne and Clinton, respectively. Fruit were harvested from treatments twice a week until disease evaluation was discontinued. Data were analyzed by analysis of variance and multiple regression.

RESULTS

The DAK provided reference points that allowed consistent rating of the plots. After using the DAK for a period of time, we used it only as a reference because memorization of damage ratings was easily accomplished. The DAK was found to be valuable for improving the abilities of others when learning to rate anthracnose (D. C. Thompson, *unpublished*).

More disease occurred at Castle Hayne than at Clinton and the higher disease severity provided better separation of the chlorothalonil rates (Fig. 2). There was more anthracnose on the cultivar Calypso than on Calico within an inoculum level ($P = 0.05$) as indicated by greater AUDPC in both environments and greater final disease at Castle Hayne. When chlorothalonil rates were increased, both AUDPC and final disease decreased at both locations. Whole-plot inoculation resulted in greater AUDPC than inoculation of the central four to six plants at Castle Hayne but did not result in greater final disease in either environment ($P = 0.05$).

Fungicide equivalents were calculated to evaluate the higher level of anthracnose resistance in Calico compared with that in Calypso. Quadratic functions were used to estimate the relationship between chlorothalonil rate and disease severity (AUDPC and final disease) and for calculating fungicide equivalents. Fungicide equivalents were calculated using anthracnose severity on Calypso, to which no chlorothalonil had been applied, as a base. The rate of chlorothalonil necessary to obtain 50% of the base value was calculated for each environment and cultivar within inoculum levels. The rate of chlorothalonil necessary on Calico was then subtracted from that for Calypso to obtain the fungicide equivalent in kilograms of chlorothalonil per week. Fungicide equivalents averaged 0.37 kg of chlorothalonil per week with a range of 0.03–0.97 (Table 1). The variances of data from the two environments were not significantly different at $P = 0.05$, and the data from both locations were combined in the analysis of variance. The disease variable used and the distribution of inoculum had a significant ($P = 0.05$) effect on the fungicide equivalent (Table 1). The base disease level did not have a significant ($P = 0.05$) influence on fungicide equivalents, using AUDPC or final disease, when the base level was placed after inoculum distribution and environment in sequential sums of squares of multiple regression.

DISCUSSION

The initial evaluation of 50 leaves per plot was very time consuming. Rating 20–30 leaves per plot and mentally averaging the ratings was thought to be a good compromise between objectivity, accuracy, and time involved. The DAK could also be used with a sampling method for larger fields.

Substantial disease severity was necessary for differences to be detected between chlorothalonil rates or inoculation levels. Higher levels of disease at Castle Hayne provided a better separation of chlorothalonil rates, initial inoculum levels, and cultivar resistance. As disease severity increased, neither rate-reducing resistance nor low fungicide rates alone were able to hold disease in check. Genetic resistance and fungicide together prevented a high percentage of disease development.

When conditions are unfavorable for disease development, the value of rate-reducing resistance is minimal since there is very little difference in the levels of disease that develop in cultivars with different levels of resistance. When conditions are favorable for moderate or high disease development, a good separation of resistance levels occurs.

Resistance that reduces the rate of disease development can most easily be described by ranking the cultivars with different levels of resistance. Enough anthracnose occurred in these tests to allow differences to be detected in cultivar resistance through the use of fungicide equivalents. The calculated fungicide equivalent values were dependent on the initial inoculum distribution and the disease variable (AUDPC or final disease) used for making the calculations but were not dependent on the environment.

The resistance of Calico compared with that of Calypso was equivalent to about 0.4 kg of chlorothalonil per week in these studies. The chlorothalonil rates currently labeled for anthracnose control are 1.21–2.49 kg/ha, which allows use of this fungicide equivalent when deciding on the rates of chlorothalonil to use on Calypso or Calico with some margin for adjustment to disease severity.

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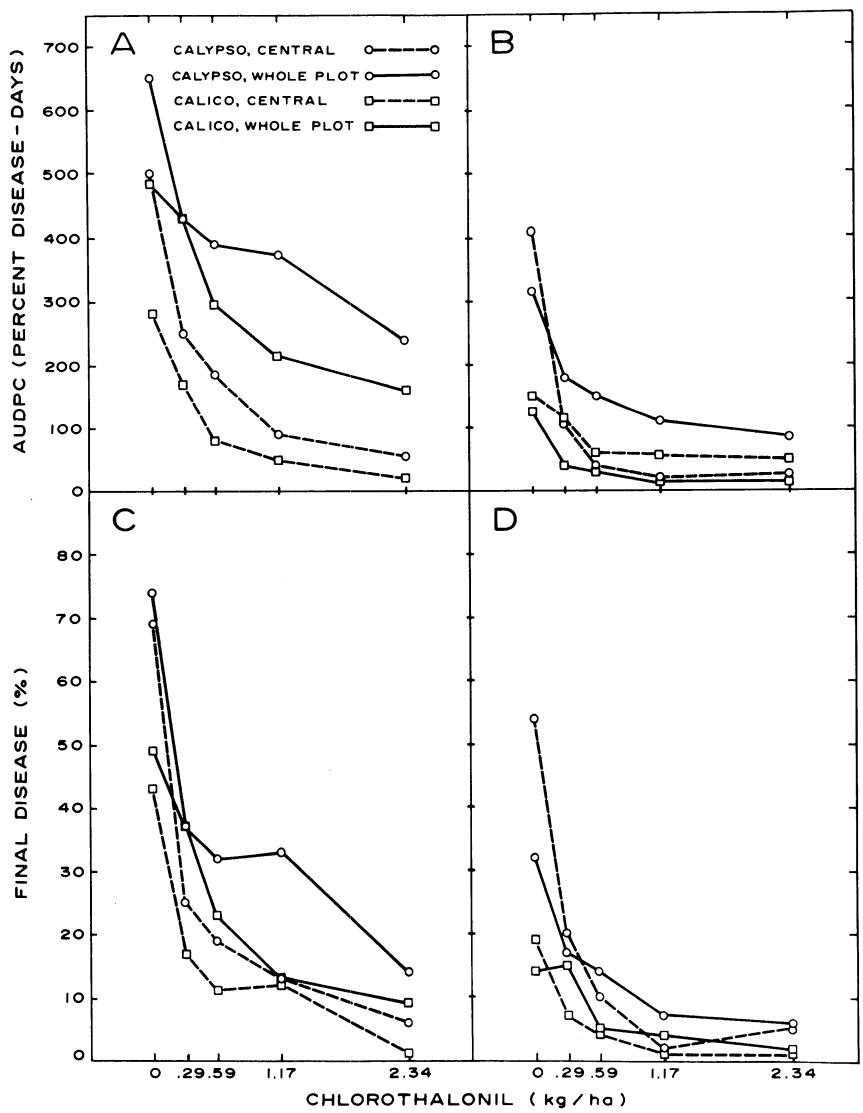

Fig. 2. Area under the disease progress curve and final disease severity of anthracnose on two cucumber cultivars with different levels of resistance at **(A and C)** Castle Hayne and **(B and D)** Clinton, NC, when increasing rates of chlorothalonil were applied weekly after inoculation of the whole plot or the central four to six plants.

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