Influence of Temperature and Leaf Wetness Period on Conidial Germination In Vitro and Infection of *Cercospora kikuchii* on Soybean

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ABSTRACT

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In vitro germination of conidia of *Cercospora kikuchii* was influenced by relative humidity. At 100% RH, 100% of the conidia germinated at all temperatures (15, 20, 25, 30, 35 C). No germination was observed below 92% RH. The temperature range tested did not alter the germination. The second fully developed trifoliate leaf of soybean (cv. Amsoy 71) was inoculated with a conidial suspension of *C. kikuchii* and then subjected to various temperatures (15, 20, 25, 30 C) and leaf wetness durations (18, 24, 30, 36 h). Disease severity was assessed 28 days later. No disease was observed at leaf wetness periods of less than 18 h or temperatures above 30 C. The optimum temperature was observed at 25 C. Disease severity increased with increasing leaf wetness period at all temperatures. The rate of increase in disease severity declined with longer leaf wetness periods. A regression model, using the arcsine-transformed disease severity values as a function of temperature (T), leaf wetness period (D), D^2, T^2, and $T \times D$, all statistically significant, described the relationship with an R^2 value of 0.81.

Cercospora leaf blight of soybeans (*Glycine max* (L.) Merr.) is caused by *Cercospora kikuchii* (Matsumoto & Tomoyasu) M. W. Gardner. The disease was first identified in Korea in 1921 (24). The first appearance in the United States was reported in Indiana in 1924 (8,9).

Three principal types of disease symptoms can be observed on soybean plants. Infection of soybean seeds leads to a purple discoloration ranging from specks to large blotches that may cover the entire surface area of the seed coat. Infected seeds are reported to have lower germination rates and result in weakened seedlings. Severe infection of soybean seedlings results in plant death, thereby causing stand reductions (23). Leaf infection is generally observed at the beginning of and throughout seed set. Reddish purple, angular to irregular lesions occur on both upper and lower leaf surfaces. Numerous infections result in necrosis of the leaf tissue and subsequent defoliation, thereby resulting in yield losses (20,26).

Previous research has been concentrated mostly on seed infection and the resulting seedling disease. The role of leaf infection, especially at the V (n) growth stages (7) of the plant, on the disease epidemiology has not been established. The objectives of this study were to determine the influence of temperature and relative humidity on conidial germination and temperature and leaf wetness duration on leaf infection of *C. kikuchii*. Results will form the basis for future investigations into the role of the leaf spot symptom in the disease epidemiology and determine optimal conditions for screening studies under controlled conditions.

MATERIALS AND METHODS

Inoculum production and culture maintenance. A population of *C. kikuchii* was obtained by randomly selecting 10 infected seeds. The seed lot was harvested at the Rock Springs Experimental Farm of the Pennsylvania State University. The fungus was maintained on clarified V8 juice with a pH of 6.0 (6,25). Five days before inoculation, fungal colonies (10 days old) were macerated with the agar substrate in a Waring blender. The resulting slurry was spread onto clarified V8 juice agar in petri dishes. These plates were incubated at 25 C under constant light (two 20-watt cool-white fluorescent lights 15 cm above the plates) for 4 days. This treatment resulted in the production of numerous conidiophores, but conidial production was absent. After 4 days, the plates were subjected to 12 h darkness/12 h light at 25 C, which induced conidial production. This procedure ensured the production of mature conidia of uniform age and size throughout the experiment. After four consecutive transfers, new fungal material from the original culture was obtained from liquid nitrogen storage; this was done to avoid loss of pathogenicity due to prolonged culture on artificial media.

In vitro germination studies. The influence of temperature and relative humidity (RH) on in vitro spore germination was investigated using the agar dish isopiestic equilibration technique described by Arauz and Sutton (2), Harris et al (10), and Alderman and Beute (1). A droplet of conidial suspension prepared as described in the inoculation study was placed on a coverslip by means of a pipette and allowed to dry. Cover slips then were placed on the bottom of a petri dish with 50 ml of water agar amended with sodium chloride suspended above it; the dish was sealed with Parafilm. Agar plates were preconditioned to the respective temperatures for 24 h before the start of the experiment. The relative humidity in these sealed petri dishes was related to the NaCl molality according to the values given by Land (16). The relative humidities were 100, 99, 98, 95, 92, and 88.5%. Additionally, a 100% RH treatment was added in which the conidial suspension was not air-dried. The petri dishes then were placed in incubators at 15, 20, 25, 30, or 35 C. After 24 h, coverslips were removed from the petri dishes and a drop of lactophenol cotton blue was added to kill and stain the fungus. Fifty randomly selected spores per coverslip were evaluated for percent germination and number of germ tubes and branching, and 10 were evaluated for the germ tube length per temperature/RH combination. The experiment was repeated three times. Incubators were randomly assigned to the temperatures.

Plant production and maintenance. Two seeds of the soybean cultivar Amsoy 71 were planted in a mixture of steam-disinfested sand/peat/loam (1:2:2, v/v/v) in 473-ml plastic containers. Plants were maintained on greenhouse benches, watered with deionized water, and fertilized biweekly with Peter's 20-20-20 (N-P-K) fertilizer. Sunlight was supplemented 12 h per day with 1,000 W high pressure sodium lamps. Approximately 3 wk after planting, plants were thinned to one plant per pot to ensure uniformity of the plant material. Greenhouse temperatures, as monitored by a hygrothermograph, ranged from 20 to 25 C throughout the study with a relative humidity of 40–60%. Plants were maintained in the greenhouse until the second trifoliate leaf was fully developed.

Inoculation. Cultures with mature conidia, produced as described above, were flooded with 15 ml of distilled water (0.5% Tween 20). The surface of the colonies was gently rubbed with a camel hair brush to dislodge conidia. The water/spore mixture then was strained through a single layer of cheesecloth to remove mycelial fragments and adjusted to 3,000 conidia per milliliter using a hemacytometer. Soybean plants were inoculated at the second trifoliate stage using a Badger airbrush (Franklin Park, IL) at 103.5×10^3 Pa pressure. The fully developed second trifoliate leaf of each plant was sprayed for 2 s from a distance of 15 cm. Leaves were allowed to dry off before the start of the treatment.

Dew chamber studies. The experiments were conducted in four Percival dew chambers (Boone, IA). Plants were subjected to air temperatures of 15, 20, 25, or 30 C and leaf wetness durations of 18, 24, 30, or 36 h in all possible combinations. In preliminary experiments, no lesions were observed outside this temperature range or below 18 h of dew. Sixteen soybean plants were used per temperature/leaf wetness duration combination. The entire experiment was conducted four times. The sequence of treatments and the assignment of dew chambers within each replicate were random. Different temperatures required unequal periods for the appearance of dew on the leaves. To avoid this variability, a misting system was engaged for 15 min after plant introduction. This ensured rapid and uniform leaf wetness without runoff.

After the temperature/dew treatment, two disks (1.6 cm diameter) were excised from randomly selected plants for each replication and fixed in lactophenol cotton blue. Percentage of spore germination and average germ tube length were determined from 20 randomly selected spores per disk. Plant surfaces were allowed to dry in the laboratory before transport to the greenhouse. Low relative humidity in the laboratory ensured rapid drying. Drying time, as estimated by visual observation, was approximately 0.5 h. The plants were maintained in a greenhouse under the same conditions as described for plant maintenance. After 4 wk, disease severity was assessed on the inoculated second trifoliate; standard area diagrams (key no. 24) were used to assess disease severity for each leaflet (12). The reported values were the average for each leaf.

Model development. The influence of postinoculation temperature and leaf wetness period on disease severity and temperature and RH on germination and germ tube growth was investigated using SAS PROC GLM (22). The model tested was of the form

$$Y = f(T, W) \quad (1)$$

in which Y is the logit or arcsine-transformed disease severity and $f(T, W)$ is a linear function of temperature (T) and leaf wetness duration (W). The terms T, T^2, T^3, W, W^2, W^3, TW, TW^2, T^2W were tested in all possible combinations. The chosen model was based on the following criteria: 1) randomness and normality of residuals; 2) significance of estimated parameters; 3) R^2 and adjusted R^2 values; and 4) R^2 value between observed and back-transformed estimated values (R^2p). Additionally, an effort was made to keep the number of terms in the model low, i.e., terms were not included in the model when, although statistically significant, their contribution to increasing the R^2 value was low.

RESULTS

Influence of temperature and relative humidity on germination in vitro. Relative humidity was the only significant variable influencing in vitro germination of the conidia when assessed after 24 h. Within the range tested, no significant effect of temperature or the temperature/RH interaction was detected. An F test indicated no significant difference in regression results among the three replications. Therefore, the data were combined. The relationship between RH and germination was best described by the equation:

$$\text{Germination} = -651.06 + 7.11\,(\text{RH}) \quad (2)$$

where RH is the percent relative humidity. The equation was significant at $P = 0.001$ and had an R^2 value of 0.58. The equation predicts no germination at or below RH of 91.6%. At the 92% RH, germination of conidia was observed only at 20 C with an average germination of 30%. The germination, averaged over temperatures, was 19.2% (with standard error of the mean = 4.9%) at 95% RH, 48.2% (4.3%) at 98% RH, 58.4% (5.4%) at 99% RH, and 56.9% (4.6%) at 100% RH. The model estimates of germination were 25.4% at 95% RH, 45.7% at 98% RH, 52.8% at 99% RH, and 59.95% at 100% RH. The germination at the 100% RH treatment, where conidia were not allowed to dry, was 100% at all temperatures.

There was no effect of either RH or temperature on the number of germ tubes produced per germinated conidium or the percentage of branched germ tubes. The number of germ tubes ranged from one to 10. Branching was observed in 16% of the germ tubes.

Influence of temperature and leaf wetness period on germination on leaf surface. On excised leaf disks, more than 90% of the conidia germinated at all dew periods (18, 24, 30, 36 h) in chambers held at 20, 25, and 30 C. At 15 C, germination was detected only at the 36-h dew period. The germination was 80%. A twisted, curvy pattern of germ tube growth precluded a precise measurement of the germ tube length. Therefore, this parameter was not statistically analyzed. In general, with the exception of the 15 C treatment, germ tubes were up to 600 μm long. At the 15 C/36 h treatment, germ tubes ranged from 50 to 100 μm. Formation of appressoria was not observed in any treatment combination.

Influence of dew period and temperature on infection. Temperature had a significant ($P \leq 0.01$) influence on disease severity (Tables 1 and 2). Significance was established by an F test on the individual regression coefficients (Table 2). The optimum temperature for infection was 25 C, independent of leaf wetness period. The ranking of temperatures in regard to disease severity was 25, 30, 20, and 15 C, most favorable to least favorable, respectively. Disease severity was, on average, twice as high at 25 C as compared with 30 C. When comparing 20 and 30 C, only small differences can be observed, with 30 C being more favorable.

Disease was observed at all leaf wetness periods at 25 and 30 C. At 20 C, no disease was observed at the 18-h leaf wetness period, and at 15 C, no disease was observed at the 18-, 24-, and 30-h leaf wetness periods.

In general, disease severity increased with increasing leaf wetness periods. The increase did not, however, follow the same pattern for the different temperatures. At 25 C, the greatest increase was from the 18- to the 24-h leaf wetness period, whereas at 20 and 30 C, marked increases (defined as an increase of at least 50% in disease severity) were observed when leaf wetness period was increased to 30 h. At all temperatures, except 15 C, further increases in leaf wetness period did not have a significant effect on disease severity. At 15 C, 36 h of leaf wetness was necessary for disease development.

Model development. A regression model based on the arcsine-transformed disease severity was chosen to model the influence

TABLE 1. The influence of leaf wetness period and temperature on the disease severity (percent leaf coverage) of *Cercospora kikuchii* on soybean (cv. Amsoy 71)

Leaf wetness period (h)	Temperature (C)							
	15		20		25		30	
	Mean[a]	SEM[b]	Mean	SEM	Mean	SEM	Mean	SEM
18	0.0	0.0	0.0	0.0	2.1	0.05	1.4	0.05
24	0.0	0.0	1.9	0.06	8.7	0.15	2.1	0.05
30	0.0	0.0	3.5	0.07	9.4	0.16	4.5	0.08
36	1.2	0.07	3.6	0.07	9.9	0.18	5.3	0.09

[a] Mean disease severity per leaf averaged over four replications ($4 \times 16 = 64$ leaves).
[b] Standard error of the mean.

TABLE 2. Estimated parameters ($P = 0.01$) from equation 3 for temperature and leaf wetness duration effects on infection of soybeans by *Cercospora kikuchii*, including coefficient of determination (R^2), R^2 adjusted for degrees of freedom (R^2_a), and R^2 adjusted for the back-transformed infection levels (R^2_p)

	Parameter estimates[a]								
	b_0	b_1	b_2	b_3	b_4	b_5	R^2	R_a^2	R_p^2
Replication 1	−1.40809	0.09461	−0.00195	0.02454	-3.74457×10^{-4}	1.73896×10^{-4}			
	(0.08828)	(0.05387)	(0.00011)	(0.00448)	7.68600×10^{-5}	7.37900×10^{-5}	0.83	0.83	0.91
Replication 2	−1.39351	0.09494	−0.00189	0.02330	-3.21460×10^{-4}	-7.31960×10^{-4}			
	(0.09056)	(0.00551)	(0.00011)	(0.00460)	7.86700×10^{-5}	7.59000×10^{-5}	0.81	0.81	0.89
Replication 3	−1.40235	0.09719	−0.00195	0.02204	-2.99326×10^{-4}	8.86980×10^{-5}			
	(0.09588)	(0.00587)	(0.00012)	(0.00488)	8.36300×10^{-5}	8.03300×10^{-5}	0.80	0.80	0.88
Replication 4	−1.44745	0.09864	−0.00195	0.02480	-3.41375×10^{-4}	4.36270×10^{-5}			
	(0.09675)	(0.00589)	(0.00012)	(0.00491)	8.43000×10^{-5}	8.04600×10^{-5}	0.80	0.80	0.87
Combined	−1.41245	0.09632	−0.00193	0.02365	-3.33822×10^{-4}	9.48020×10^{-5}			
experiments	(0.04615)	(0.00281)	(0.00005)	(0.00234)	4.01900×10^{-5}	3.58580×10^{-5}	0.81	0.81	0.89

[a]Estimated parameters for equation 3 corresponding to intercept, T, T^2, W, W^2, T × W. Numbers in parentheses correspond to the parameters' standard deviation.

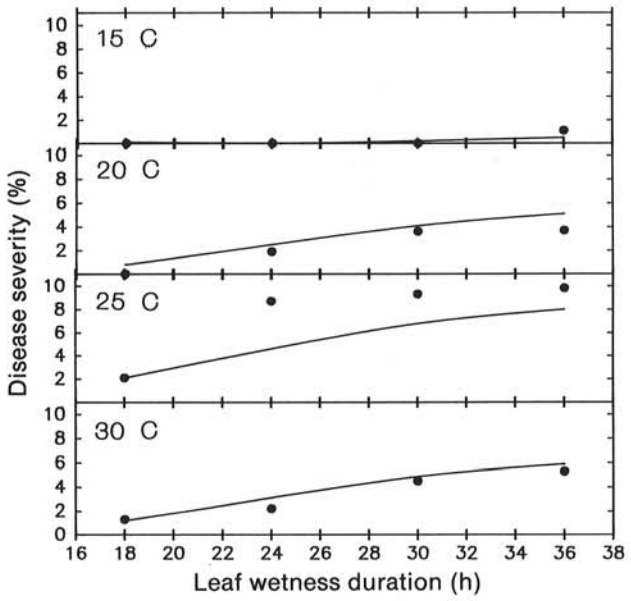

Fig. 1. Prediction of the disease severity (percent leaf coverage) of *Cercospora kikuchii* for various combinations of temperature and leaf wetness duration. The solid lines represent equation 3 based on the pooled data. The plotted points were averages of observations made on four replications (64 plants).

of temperature and leaf wetness duration. No difference was detected between the regression of each replication, based on an F test of full and reduced regression models (19). The data were therefore combined. The equation for the model was

$$\text{arcsine}\ (y) = b_0 + b_1T + b_2T^2 + b_3W + b_4W^2 + b_5TW \quad (3)$$

in which y is the percent disease severity and $b_0 \ldots b_5$ are the regression coefficients, all of which were significant at $P = 0.01$, with the exception of b_5, which was significant at $P = 0.014$ (Table 2). The equation described a relationship in which both temperature and dew period had a significant influence on disease severity. The parameter estimates for the squared terms (b_2 and b_4) were negative. This resulted in a smaller increase in disease severity with increasing dew periods, eventually leading to an asymptote. The statistical significance of the interaction parameter showed that the change in y with a change in temperature was not the same for all dew periods or vice versa. The R^2, adjusted R^2, and R^2p values for the combined data were 0.81, 0.80, and 0.89, respectively.

The estimated parameters of equation 3 were used to calculate disease severities for the appropriate temperature/dew period combinations (Fig. 1). An increase in disease severity was seen at all temperatures with increasing dew period. The behavior of the curve, as with the observed disease values, showed an asymptotic behavior at the longer dew periods, with the exception of the 15 C temperature treatment. The optimum temperature was 25 C, regardless of the duration of the dew period. The model estimated disease severities at 15, 20, and 30 C closely. It underestimated disease severity at the 24–30 h dew period at 25 C. Manipulation of the interaction term corrected this but led to overprediction for the other temperatures.

DISCUSSION

Temperature and leaf wetness period have a strong influence on the disease severity defined as percent leaf area. Disease severity, as compared with lesion number, was chosen as the response variable because separation into individual lesions was sometimes difficult. Additionally, the very slow expansion rate of lesions on young, green tissue minimizes assessment errors caused by confounding infection and lesion growth.

Preliminary data by Martin and Walters (17) described similar minimum and maximum temperatures for infection. They observed an optimum temperature (20–24 C) slightly lower than the one observed in this study at 25 C. In the present study, if Y (disease severity) is differentiated with respect to temperature and the derivative set equal to 0 (18), the resulting optimum temperature is 25.3 C. Mathematical determination of the optimum period of leaf wetness through differentiation and setting the resulting equation to 0 resulted in an optimum leaf wetness period of 78.9 h. Because this value is outside the tested range, its validity is questionable. However, the small increase in disease severity with increased leaf wetness from 30 to 36 h at 20, 25, and 30 C (0.1, 0.5, and 0.8%, respectively) suggests further increases in leaf wetness periods to be of little importance for disease epidemics. Leaf wetness periods greater than 24 h function as a threshold, i.e., they determine if infection takes place, whereas temperature in the range of 15–30 C functions quantitatively, i.e., it determines the speed and range of symptom development. When temperature becomes limiting as in the 15 C treatment, extended leaf wetness periods have a potential for compensation. This also can be seen in the significant interaction parameter in equation 3.

Experiments involving *C. kikuchii* have concentrated mainly on the pod infection or leaf infection late in the season (11,18,21, 27). However, the fungus overwinters on infected soybean debris left on the soil surface (13) and sporulates readily in the spring. During this time, conidia are readily trapped in large numbers using volumetric spore trap (C. Orth and W. Schuh, *unpublished data*). Favorable environmental conditions, as determined through this study, are common during spring, but symptom expression of foliar infection under field conditions generally becomes visible at the beginning of and through full seed set (26). This opens the question to the role in the disease epidemiology of leaf infection

during the early plant developmental stages. Abundant sporulation, as determined through volumetric spore trapping, can be detected throughout the growing season, even though disease severities assessed in field experiments during the same time period were relatively low (Orth and W. Schuh, *unpublished data*). Latent or symptomless infections have been observed in soybeans when treated with paraquat (4,14). It is unclear, however, if these latent infections would support sporulation without the paraquat treatment, i.e., latent infections could be a resistance mechanism of the plant. This question is currently being addressed.

The model developed was satisfactory for describing the relationship between disease severity and the variables temperature and humidity. A high proportion of variability was accounted for by the model as determined by the coefficient of determination based on the combined data ($R^2 = 0.81$) and the high agreement between observed, estimated, and back-transformed severity values ($R^2 = 0.89$). Although the model closely predicted the disease severity at 15, 20, and 30 C, it underestimated the disease severity for the 24–36 h leaf wetness periods at 25 C. This should not be due to the choice of a particular modeling approach, i.e., multiple regression. Predictive models based on the use of growth functions (3,15) had similar problems and are not inherently superior for modeling purposes.

The temperature optimum for infection seems to be independent of the temperature requirements for germination. After 24 h, the time necessary for infection to occur in all but the 15 C treatment, no difference was detected in percent germination, germ tube number, and branching in the in vitro experiment. When spores were not subjected to drying, 100% germination occurred at all temperatures. In the leaf wetness period study, however, the 15 C temperature treatment delayed germination up to the 36 h leaf wetness period. The ability of the fungus to germinate without a water film suggests the possibility of infection at high RH without leaf wetness, especially since no appressoria were observed in the leaf disk study. Sporulation and dispersion in the field is not dependent on rainfall events (C. Orth and W. Schuh, *unpublished data*). This question will be addressed in future studies.

Germ tube length, even though not statistically analyzed in this study due to the irregular growth pattern, does not seem to be a determining factor in the infection process. Germ tubes were generally very long (up to 600 μm), and length was not influenced by temperature, except in the 15 C treatment.

No infection structures such as appressoria or infection pegs were observed in this study. Evidence of direct penetration was found by Chen et al (5). They observed direct penetration of the seed through pores in the seed coat without the formation of additional structures. No penetration of stomata by germ tubes was observed in this study. Scanning electron microscopy indicated the possibility of direct penetration of the leaf surface. The number of germ tubes investigated was probably too low to draw a definite conclusion.

The model developed in this study can be used to determine optimum infection conditions for experiments relying on artificial inoculation under controlled conditions. Results also point to the importance of infections of young plants, and the influence of high RH, in the disease epidemiology under field conditions, especially in regard to the source of the airborne conidia.

LITERATURE CITED

1. Alderman, S. C., and Beute, M. K. 1986. Influence of temperature and moisture on germination and germ tube elongation of *Cercospora arachidicola*. Phytopathology 76:715-719.
2. Arauz, L. F., and Sutton, T. B. 1989. Influence of temperature and moisture on germination of ascospores and conidia of *Botryosphaeria obtusa*. Phytopathology 79:667-674.
3. Carisse, O., and Kushalappa, A. C. 1990. Development of an infection model for *Cercospora carotae* on carrot based on temperature and leaf wetness duration. Phytopathology 80:1233-1238.
4. Cerkauskas, R. F., and Sinclair, J. B. 1980. Use of paraquat to aid detection of fungi in soybean tissues. Phytopathology 70:1036-1038.
5. Chen, M. D., Lyda, S. D., and Halliwell, R. S. 1979. Infection of soybeans with conidia of *Cercospora kikuchii*. Mycologia 71:1158-1165.
6. El-Gholl, N. E., Alfieri, S. A., Ridings, W. H., and Schoulties, C. L. 1982. Growth and sporulation in vitro of *Cercospora apii, Cercospora arachidicola, Cercospora kikuch* and other species of *Cercospora*. Can. J. Bot. 60:862-868.
7. Fehr, W. R., Caviness, C. E., Burmood, D. T., and Pennington, J. S. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Sci. 11:929-931.
8. Gardner, M. W. 1925. Indiana plant diseases, 1924. Proc. Indiana Acad. Sci. 35:237-257.
9. Gardner, M. W. 1926. Indiana plant diseases, 1925. Proc. Indiana Acad. Sci. 36:231-247.
10. Harris, R. F., Gardner, W. R., Adebayo, A. A., and Sommers, L. E. 1970. Agar dish isopiestic equilibration method for controlling the water potential of solid substrates. Appl. Microbiol. 19:536-537.
11. Hepperly, P. R. 1984. Purple seed stain incidence among soybean cultivars and between seasons in Puerto Rico. J. Agric. Univ. P.R. 68:87-99.
12. James, W. C. 1971. An illustrated series of assessment keys for plant diseases, their preparation and usage. Can. Plant Dis. Surv. 51:39-65.
13. Jones, J. P. 1968. Survival of *Cercospora kikuchii* on soybean stems in the field. Plant Dis. Rep. 52:931-934.
14. Kmetz, K. T., Ellett, C. W., and Schmitthenner, A. F. 1979. Soybean seed decay: Sources of inoculum and nature of infection. Phytopathology 69:798-801.
15. Lalancette, N., Ellis, M. A., and Madden, L. V. 1988. Development of an infection efficiency model for *Plasmopara viticola* on American grape based on temperature and duration of leaf wetness. Phytopathology 78:794-800.
16. Lang, A. R. 1967. Osmotic coefficients and water potential of sodium chloride solutions from 0 to 40 C. Aust. J. Chem. 20:2017-2023.
17. Martin, K. F., and Walters, H. J. 1982. Infection of soybean by *Cercospora kikuchii* as affected by dew temperature and duration of dew periods. (Abstr.) Phytopathology 72:974.
18. Miller, W. A., and Roy, K. W. 1982. Effects of benomyl on the colonization of soybean leaves, pods, and seeds by fungi. Plant Dis. 66:918-920.
19. Neter, J., and Wasserman, W. 1974. Applied Linear Statistical Models. Richard D. Irwin, Inc., Homewood, IL.
20. Ross, J. P. 1975. Effect of overhead irrigation and benomyl sprays on late-season foliar diseases, seed infection, and yields of soybean. Plant Dis. Rep. 59:809-813.
21. Roy, K. W., and Abney, T. S. 1976. Purple seed stain of soybeans. Phytopathology 66:1045-1049.
22. SAS User's Guide: Statistics. 1985. Version 5 ed. SAS Institute Inc., Cary, NC.
23. Sinclair, J. B., and Backman, P. A., eds. 1989. Compendium of Soybean Diseases. 3rd ed. American Phytopathological Society, St. Paul, MN.
24. Suzuki, K. 1921. Studies on the cause of 'Shihan' of soybeans. Chosen Nakaiho 16:24-28.
25. Vathakos, M. G., and Walters, H. J. 1979. Production of conidia by *Cercospora kikuchii* in culture. Phytopathology 69:832-833.
26. Walters, H. J. 1980. Soybean leaf blight caused by *Cercospora kikuchii*. Plant Dis. 64:961-962.
27. Wilcox, J. R., and Abney, T. S. 1973. Effects of *Cercospora kikuchii* on soybeans. Phytopathology 63:796-797.