RESISTANCE COMPONENTS AND TEMPERATURE EFFECTS ON EPIDEMIOLOGY OF *CERCOSPORA ARACHIDICOLA* HORI ON PEANUTS

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PREFACE

Different techniques have been used to evaluate peanut resistance to Cercospora leafspot. Using solely the number of lesions produced per leaflet, or the number of conidia produced per mm^2 of necrotic lesion, or lesions size as a measurement for resistance to early leafspot has major disadvantages. The purpose of this study is to evaluate the sporulating potential per leaflet, as a measure of resistance. The factors or components determining the sporulating potential were determined using the detached leaf culture technique. The effect of temperature epidemiological implications on sporulation are also discussed.

I wish to express my profound appreciation to my major advisor, Dr. Hassan A. Melouk, for his unlimited assistance and guidance throughout this study. Appreciation is also expressed to other committee members, Dr. Kenneth Conway, Dr. Francis Gough, and Dr. Dallas F. Wadsworth for their invaluable suggestions and assistance in the preparation of the final manuscript.

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CHAPTER I

INTRODUCTION

This thesis is comprised of two manuscripts written in a format that will facilitate immediate submission to a national scientific journal. These manuscripts are presented as chapters in this thesis, and each is complete in itself without additional supporting materials. The manuscript entitled, 'Sporulating potential of Cercospora arachidicola as a criterion for screening peanut genotypes for leafspot resistance' (Chapter II), was to evaluate different methods for measuring peanut resistance to C. arachidicola and propose a method that incorporates these different resistance components, and eliminates the disadvantage of using solely one parameter. The manuscript entitled 'Resistance components of peanuts to Cercospora arachidicola' (Chapter III), was to determine how good an estimator the different resistance components were in estimating the sporulating potential. Both manuscripts are written to specifications of PHYTOPATHOLOGY.

Approval for presenting the thesis in this manner is based upon the Graduate College's policy of accepting a thesis written in manuscript form and is subject to the Graduate College's approval of the major professor's request for a waiver of the standard format in a letter dated 9 February 1977.

CHAPTER II

SPORULATING POTENTIAL OF *CERCOSPORA ARACHIDICOLA* AS A CRITERION FOR SCREENING PEANUT GENOTYPES FOR LEAFSPOT TESISTANCE

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ABSTRACT

The sporulating potential of *Cercospora arachidicola* on peanut is defined as the total number of conidia produced on an infected peanut leaflet after 96 hours of incubation at 25 ± 1 C under continuous light (800 lux) and 100% relative humidity. Sporulating potential was determined on seven peanut genotypes, using a detached leaf culture technique. This determination was found to be a better measurement of resistance than solely the number of lesions per leaflet, the number of conidia produced per mm^2 of necrotic lesion, or total necrotic lesion area per leaflet. Genotypes that did not differ in total number of lesions per leaflet, and number of conidia produced per mm^2 of lesion were significantly ($P = .05$) different in number of conidia produced per leaflet. Increase in necrotic lesion area increased the sporulating potential. No conidia were recovered from some of the genotypes even after prolonged incubation of infected leaflets. There was a significant linear correlation between necrotic lesion area and total lesions per leaflet on Comet, P.I. 109839, Florunner and Tamnut 74. No difference was obtained in percent defoliation between genotypes.

Additional key words: Arachis hypogaea, Arachis sp., Early leafspot, disease resistance, epidemiology.

INTRODUCTION

Early leafspot caused by the fungus Cercospora arachidicola Hori, is one of the most economically important diseases of peanuts (Arachis hypogaea L.) world wide.

Control of C. arachidicola has been aimed at reducing the initial inoculum and/or subsequent inoculum production either through chemical control, crop rotation, sanitation, and other cultural practices. Chemicals have provided the best control (2, 3, 13, 15), however, the development of benomyl tolerant strains of Cercospora and Cercosporidium (5, 11, 17) indicates a reduction in the usefulness of benomyl and related compounds, and makes the search for resistant varieties imperative.

All known cultivars are susceptible in varying degrees to C. arachidicola (1). Various workers have identified potential peanut germplasm useful in breeding programs (4, 8, 9, 18). Evaluation of Cercospora resistance has been made on the basis of lesion counts, and the estimation of the degree of sporulation using an index scale set by the investigator. Based solely on the number of lesions produced per leaflet, a peanut genotype may be disqualified from a breeding program, even though further investigation may reveal little or no sporulation on these lesions.

The detached leaf culture technique (12) developed for rapid screening of peanut genotypes for resistance to leafspot has been correlated significantly with field evaluations (6). In this paper, using the detached leaf culture technique (12), the sporulating potential of C. arachidicola on peanut leaflets, as determined by: (a) number of lesions per leaflet, (b) lesion area, and (c) number of conidia produced per mm^2 of lesion

area, is being proposed as a criterion for evaluating peanut genotypes for resistance to early leafspot.

MATERIALS AND METHODS

Seven peanut genotypes were selected for this study. Spanish genotypes, Tamnut 74, and Comet; Virginia genotypes, Florunner and P.I. 109839, a plant introduction from Venezuela described as resistant to C. arachidicola (18); wild species, P.I. 276233 [(GK 10596), A. sp., section RHIZOMATOSAE], 276235 [A. chacoense Krap. & Greg. (nomen nudum) section ARACHIS], one hybrid M143 (P.I. 338280 X P.I. 276235).

Tamnut 74, Comet, Florunner and P.I. 109839 were grown from captan-ethrel-treated seeds. Seeds were planted in 16 cm diameter plastic pots, containing a 2:2:1 mixture of soil, sand and a finely shredded peat. The other genotypes were grown from shoot cuttings taken from greenhouse grown plants several months old. Pots were placed on a bench in a greenhouse under conditions favourable to the growth of peanuts.

The third expanded leaf was detached from test plants 6 to 8 weeks old for seed grown plants, and several months old for plants grown from cuttings. Detached shoots were used instead of leaves from P.I. 276235, P.I. 276233, and M143 because of short petioles. Petioles or shoots were inserted into test tubes (16 X 150mm) containing Hoagland's solution (10) and supported by foam plugs (12). Design of the experiment was a randomized block design with genotypes as treatments, and replicates as blocks. There were a total of 16 leaves or shoots per treatment representing four replicates.

The single spore isolate of C. arachidicola used in this experiment was obtained from infected plants grown in greenhouse at Stillwater, Oklahoma. Conidia were prepared for inoculum as described by Smith (16),

except that they were suspended (2 X 10^4 conidia/ml) in an emulsion of Amway (Amway Corp., Michigan 49301) all purpose adjuvant (2 drops/100 ml H_2O). Both surfaces of the leaflets were misted with the conidial suspension using a DeVilbiss No. 152 atomizer (The DeVilbiss Company, Somerset, PA 15501). Test tubes in racks were placed in fabricated clear polyethylene moisture chambers (one replicate/chamber), on greenhouse benches. Relative humidity was recorded at 100% with a hygrothermograph placed in the chamber, and maintained by wetting burlap bags placed at the bottom of each chamber. Temperature inside the chamber ranged from 21 - 32 C. The lowest temperatures were recorded during the night and the higher temperatures during the day. Hoagland's solution in test tubes was replenished as needed.

Number of lesions per leaflet were counted three weeks after inoculation. Where shoots were used, only leaves corresponding to the third expanded leaf at the time of detachment were read. Leaflets with lesions were incubated in petri-dish moist chambers for 96 hours under continuous light (800 lux), provided by 40w Cool White Econ-o-watt florescent, at 25 ± 1 C. Lesions on leaflets were examined under a disecting microscope for sporulation. Conidia were washed from the surface of leaflets with 2 ml distilled water for each four leaflets, and the number of conidia in the suspension were determined using a hemacytometer. Lesions on the leaflets were excised and the surface area determined with a Li-Cor Model 3100 (Lambda Instruments Corporation, Lincoln, NE 68504) area meter. The sporulating potential of C. arachidicola was then calculated.

RESULTS

Mean number of leafspot lesions per leaflet (Table 1) was signifi-

cant (P = .01) among peanut genotypes. Lesions on Comet, Tamnut, Florunner, and P.I. 109839 appeared 11 to 13 days after inoculation while on P.I. 276235, P.I. 276233 and M143 were noticed 13 to 15 days after inoculation. There was no difference in mean number of lesions per leaflet between Florunner, P.I. 278235, P.I. 276233, and M143 (Table 1).

Conidia produced per mm^2 of necrotic area was significant (P = .01), among peanut genotypes, and ranged from 0 to 674 (Table 2). No conidia were recovered from lesions on P.I. 276235, P.I. 276233, or M143 even after incubating the leaflets for 10 to 13 days, at which time leaflets started to deteriorate. Sporulation of C. arachidicola on Tamnut 74 was signigicantly higher (P = .01) than on Florunner, and also higher on Comet and P.I. 109839 (P = .05) than on Florunner. However, there was no difference in sporulation between Tamnut 74, Comet or P.I. 109839.

A two-way analysis was performed on the data obtained from the peanut on which sporulation of C. arachidicola occured, (Table 3). The mean necrotic lesion area and conidia produced per leaflet were significantly higher on Tamnut 74 and Comet than on P. I. 109829 or Florunner. There was no difference between Tamnut 74 and Comet in number of conidia produced per leaflet. The mean lesion area per leaflet was higher (P = .05) on Comet than on any other genotype, except Tamnut 74.

Positive and significant linear correlation between necrotic lesion area and number of lesions per leaflet was found on Tamnut 74, Comet, Florunner, and P.I. 109839 (Fig 1). Higher correlation values were obtained on Comet, Florunner, and P.I. 109839 (P = .001) than on Tamnut 74 (P = .05).

Leaflet defoliation of C. arachidicola infected peanut genotypes was scored 21 days after inoculation. There was no defoliation on Comet,

Florunner and P. I. 276235. Defoliation of 17.2, 10.9, 7.8 and 4.7% was noted on P. I. 276233, M143, Tamnut 74 and P. I. 109839, respectively; however, defoliation was not significant among them.

DISCUSSION

Greenhouse screening for Cercospora resistance using the detached leaf culture technique has been shown to be useful, however, due to the large variation involved with different methods of disease estimation, no one method is adequate. Visual estimation may be subjective in nature as stated by Pedrosa (14). The sporulating potential of C. arachidicola as defined in this paper, represents an important parameter for a more comprehensive estimation of leafspot severity, that is determined by the number of necrotic lesions per leaflet, the necrotic lesion area per leaflet, and the number of conidia produced per mm^2 of necrotic lesion. There was no difference in mean number of lesions or conidia produced per mm^2 of lesion between Tamnut 74, Comet and P.I. 109839, however, total number of conidia produced per leaflet was different ($P = .05$) between Tamnut 74 and P.I. 109839, probably due to differences in lesion area. P.I. 109839 has been reported as having a high degree of resistance (18) to C. arachidicola, however, Foster et al (7) recently reported high production of conidia per lesion and per mm^2 on P.I. 109839. These results are comperable to those obtained in this study. Conidia per mm^2 produced on P.I. 109839 after 96 hours incubation were 2 - 3 folds of those obtained by Foster et al (7) who incubated Cercospora lesions for 48 hours.

Florunner has not been reported as resistant and the low sporulation and fewer number of lesions per leaflet obtained in this study indicates a need for further investigation. It is, however, suspected that the low

sporulation potential measured on Florunner was due largely to the immaturity of some of the lesions at the time leaflets were harvested. Genotypes with low sporulating potential may have a stabilizing effect on disease development by reducing the rate of epidemic increase (19) under field conditions.

The value of the correlation coefficient obtained when regression analysis between number of lesions per leaflet, and necrotic lesion area is performed, gives a measure of the variation in lesion size and lesion maturity for each genotype (Fig.1). The variation was more on Tamnut 74 ($P = .05$) than on Comet, Florunner, or P.I. 109839 ($P = .001$). Inspite of the higher variation on Tamnut 74, the sporulating potential recorded on the genotype compares favorably with its susceptibility level to C. arachidicola.

Leafspot symptoms appeared on Tamnut 74, Comet, Florunner, and P.I. 109839, 11 to 14 days after inoculation when the detached leaf culture technique was used, however, symptoms on whole plants were observed 7 to 10 days after inoculation. This difference in the latent period may have influenced the defoliation results. Defoliation results using the detached leaf culture technique cannot be correlated directly with whole plant inoculation, as defoliation of one or two leaflets from an infected leaf corresponds to 25 or 50% defoliation, respectively. They can only be evaluated in their own context, and as they compare with results from none inoculated controls.

Using the total number of conidia produced on an infected leaflet as a measurement for resistance eliminates some of the major disadvantages of using solely the number of necrotic lesions per leaflet, or the number of conidia produced per mm^2 of necrotic lesion, as criteria in evaluating peanuts for resistance to C. arachidicola.

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TABLE 1. Mean number of necrotic lesions per leaflet on different peanut genotypes 21 days after inoculation with Cercospora arachidicola

Genotype	Replicate				Mean 2/
	1	2	3	4	
Tamnut 74	27.1	11.9	3.4	4.5	11.7 a
Comet	15.1	14.1	6.7	8.8	11.2 a
P.I. 109839	7.9	12.8	3.9	3.8	7.1 ab
Florunner	2.5	0.7	4.1	4.9	3.1 b
P.I. 276233	2.3	1.1	0.2	0.1	0.9 b
P.I. 276235	1.6	0.1	0.1	0.0	0.5 b
M143 1/	0.6	0.4	0.1	0.2	0.3 b

1/ A hybrid between P.I. 338280 X P.I. 276235.

2/ Means followed by the same value are not significantly different (P = .05), using Duncan's new multiple range test.

TABLE 2. Mean number of conidia of C. arachidicola produced per mm^2 of necrotic lesion area 21 days after inoculation

Peanut Genotype	Replicate				Mean 2/
	1	2	3	4	
Tamnut 74	492	312	743	1150	674 a
Comet	389	227	801	1023	610 a
P.I. 109839	578	136	871	886	618 a
Florunner	191	173	191	598	288 b
P.I. 276233	0	0	0	0	0 c
P.I. 276235	0	0	0	0	0 c
M143 1/	0	0	0	0	0 c

1/ A hybrid between P.I. 338280 X P.I. 276235

2/ Means followed by the same value are not significantly different (P = .05), using Duncan's new multiple range test.

TABLE 3. Mean of necrotic lesion area and number of C. arachidicola conidia produced per leaflet in different peanut genotypes

Genotype	Necrotic Lesion area per leaflet mm^2	Conidia produced per mm^2 of necrotic area	Conidia produced per leaflet
Comet	44.7	610	24809
Tamnut 74	28.7	674	19322[1/]
P.I. 109839	11.1	618	6843
Florunner	9.2	288	2661
LSD (.05)	16.6	233	9505
(.01)	23.8	335	13657

[1/] Mean of 64 leaflets.

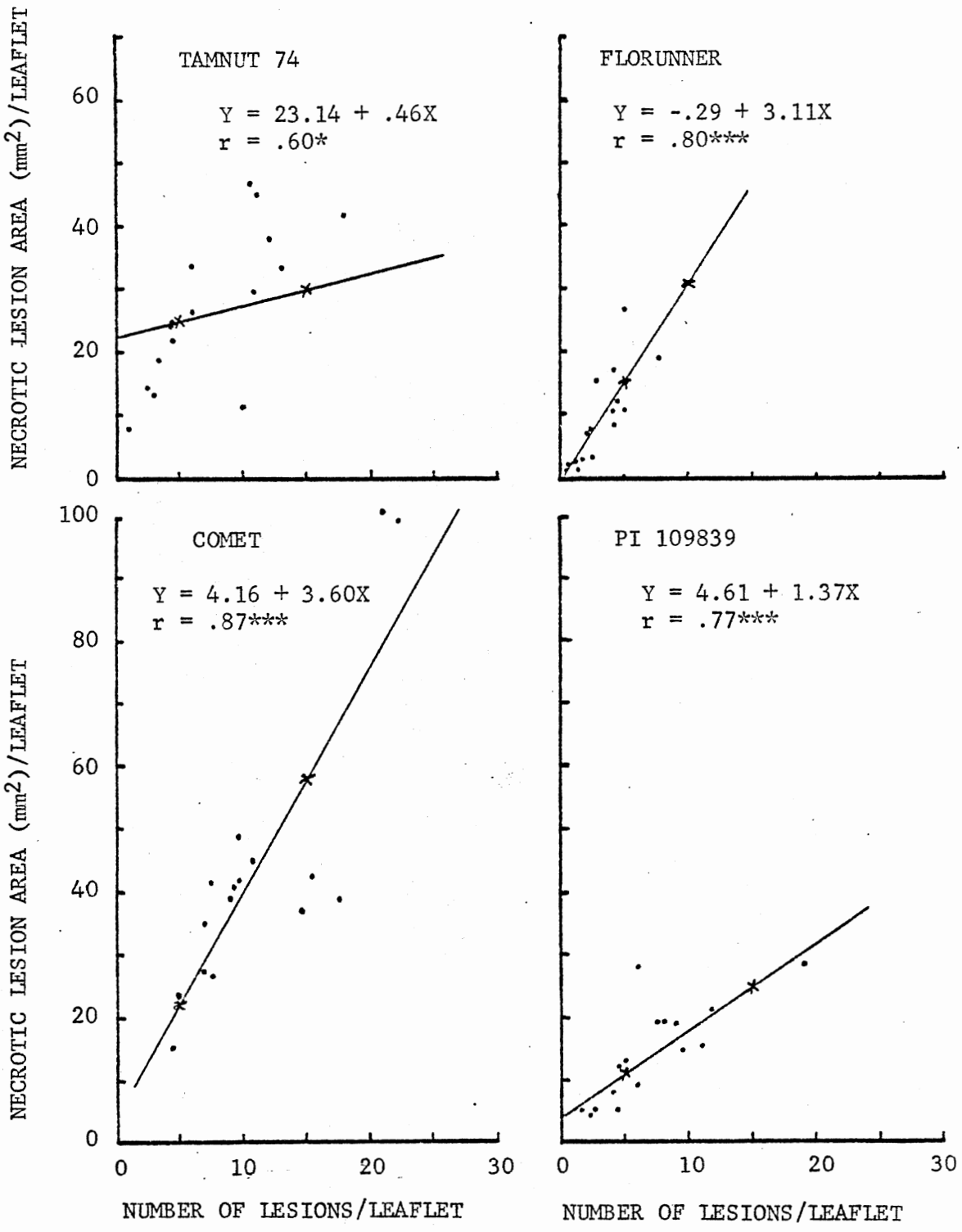

Fig. 1. Linear regression between necrotic lesion area (mm^2) per leaflet and number of lesions per leaflet in peanut caused by Cercospora arachidicola

CHAPTER III

RESISTANCE COMPONENTS OF PEANUTS TO *CERCOSPORA ARACHIDICOLA*

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ABSTRACT

The effect of some resistance components of peanuts (*Arachis hypogaea*) to *Cercospora arachidicola* on the number of conidia produced per leaflet was determined. A multiple linear regression model with conidia/leaflet as predictor variable, and conidia/mm^2 of necrotic lesion, total lesions/leaflet, necrotic lesion area and mature lesions per leaflet as independent variables was significant (R^2 = .956, P = .01). Genotypic effects were found to influence conidia/mm^2 and total leasions/leaflet. Temperature influenced sporulation and conidial germination. Sporulation of *C. arachidicola* was optimum at 30 C, while the highest germination was recorded at 25 and 30 C after 6 and 12 hours incubation. Light increased the length of conidia 1.8 times compared to dark.

Additional key words: Disease resistance, *Cercospora arachidicola* Peanut.

INTRODUCTION

The number of propagules produced by a pathogen is one of the chief factors determining epidemic development in plant diseases. Though the

amount of the inititial inoculum is important in disease development, no one portion of the disease cycle exerts a greater influence on epidemic increase than the production of inoculum for subsequent infection. Any effort to relate rate of epidemics (18) to conidial production must be through a build up of inoculum.

Peanut genotypes are susceptible to Cerospora arachidicola Hori in varying degrees (2). Resistance or lack of it may be manifested in, a) the number of lesions produced; b) the size of lesions; c) the duration of the latent period; and d) the rate of lesion development.

The number of propagules produced by a pathogen on its host reflects the pathogenicity of the pathogen, and is the sum of all the components of resistance mechanisms in the host (6). Sporulation of C. arachidicola on different peanut genotypes is influenced by a combination of the above resistance factors. However, temperature and relative humidity (5, 7) also influence the number of conidia produced on Cercospora lesions. Miller (10) obtained optimum growth of C. arachidicola at 25 to 32 C. A correlation between disease and periods of high relative humidity during which temperatures were in the 20's (C) was reported by Jensen and Boyle (5). Lyle (7) obtained the greatest number of conidia during a period of abundant rainfall and high minimal (22 C) and maximal (35 C) temperatures. Abdou (1) found that light was not required for sporulation in culture of C. arachidicola but was necessary for Cercosporidium personatum.

Primary infection of C. arachidicola are by spores blown or splashed on leaves (13) and subsequent infection by conidia borne by wind, rain, insects, and use of mechanical equipment (3, 15).

Progress in identifying and evaluating resistance in peanut genotypes to C. arachidicola has been slow due to the disease rating methods that have been used, and the difficulty of creating satisfactory epiphytotics. The objectives of this investigation were to, i) determine the percent lesion maturity as indicated by the presence of stromata; ii) to determine the rate of lesion increase as estimated by the lesion diameter; iii) examine the epidemiological implications by analyzing spore viability and pathogenicity data; and iv) determine the effect of temperature on sporulation and conidial germination of C. arachidicola.

MATERIALS AND METHODS

Nine peanut genotypes were selected for this study. Spanish genotypes, Tamnut 74, and Comet; Virginia genotypes, Florunner and P.I. 109839, a plant introduction from Venezuela described as resistant to C. arachidicola (2); wild species, P.I. number 276233 [(GK 10596), A. sp., section RHIZOMATOSAE], 276235 (A. chacoense Krap. & Greg. (nomen-nudum) section ARACHIS, and 338280 (A. sp., section ARACHIS: two hybrids M143 (P.I. 338280 X P.I. 276235) and M213 [Chico X (P.I. 338280 X P.I. 276235)].

Tamnut 74, Comet, Florunner, P.I. 109839 and P.I. 338280 were grown from captan-ethrel treated seeds. Seeds were planted in 16 cm diameter plastic pots, containing a 2:2:1 mixture of soil, sand, and finely shredded peat. The other genotypes were grown from shoot cuttings taken from greenhouse grown plants several months old. Pots were placed on a bench in a greenhouse under conditions favorable to the growth of peanuts. The experiment was conducted using the detached leaf culture technique (9).

The third expanded leaf was detached from test plants 6 to 8 weeks old grown from seeds, except P.I. 338280, and several months old plants (M213) grown from cuttings. Detached shoots were used instead of leaves from P.I. 276235, P.I. 276233, M143, and P.I. 338280 because of short petioles. Petioles or shoots were inserted into test tubes (16 X 150mm) containing Hoagland's solution (4) and supported by foam plugs (9). Design of the experiment was a completely randomized block design with genotypes as treatments, and replicates as blocks. There were a total of 12 leaves or shoots per treatment representing three replicates.

Single spore isolate of C. arachidicola used in this experiment was obtained from infected plants grown in greenhouse at Stillwater, Oklahoma. Conidia were prepared in the manner described by Smith (12) except that they were suspended (2.2×10^4 conidia/ml) in an emulsion of Amway (Amway Corp., Michigan 49301) all purpose adjuvant (2 drops/100 ml H_2O). Both surfaces of the leaflets were misted with the conidial suspension using a DeVilbiss No. 152 atomizer (The DeVilbiss Company, Somerset, PA 15501). Test tubes in racks were placed in fabricated clear polyethylene moisture chambers (one replicate/chamber), under greenhouse benches. Relative humidity was maintained at 100% by wetting burlap bags placed at the bottom of each chamber. Temperature inside the chamber ranged from 22 - 32 C within the first two weeks, and 20 - 26 C during the third and final week after inoculation. The lower temperatures were recorded during the night and the higher temperatures during the day. Hoagland's solution in test tubes was replenished as needed.

Number of lesions and mature lesions with stromata were recorded three weeks after inoculation. Where shoots were used, only leaves corresponding to the third expanded leaf at the time of detachment were

evaluated. Lesions with stromata were counted using a dissecting microscope. Leaflets were incubated in petri-plate moist chambers for 96 hr under continous light (800 lux), provided by 40w Cool White Econ-o-watt florescent, at 25 ± 1 C. Lesions were examined under the binocular microscope for sporulation. Conidia were washed from the surfaces of leaflets with 2 ml distilled water for each four leaflets, and the number of conidia in the suspension were determined using a hemacytometer. The conidial suspension was saved and used in the viability and pathogenicity tests. Lesion diameter of the large lesions was measured with a calipers while an ocular micrometer was used to measure the diameter of the small lesions. Necrotic lesions were excised from Tamnut 74, Comet, Florunner and P.I. 109839, and the surface area determined with a Li-Cor model 3100 (Lambda Instrments Corporation, Lincoln, Nebraska 68504) area meter.

Conidial viability was determined using conidial suspensions saved from the detached leaf experiment. Conidia were incubated in slide depression wells placed in petri-dish moist chambers at 25 ± 1C, under continous light (800 lux) for 24 hr. Germinating conidia were then counted under the microscope.

In the pathogenicity test, shoots collected from 6 to 8 week old Tamnut 74 plants were inoculated with the conidial suspension saved from the detached leaf experiment in a manner described earlier except for some modifications of the method which will be outlined in the results.

To determine the effect of temperature on conidial production of C. arachidicola on mature lesions, 6 week old Tamnut 74 plants were inoculated in a manner as above. Pots were placed in clear polyethylene chambers on greenhouse bench and the relative humidity maintained at 100% as in the detached leaf experiment. Temperature in the chambers ranged from 21-32 C.

Plants were removed from the chambers as soon as distinct Cercospora lesions were noticed (10-13 days after inoculation), and placed on greenhouse bench. Lesions were allowed to develop at a low relative humidity of about 70%, to preclude conidial production of lesions. Leaflets with few (3 - 8) distinct and mature lesions were collected, and randonly placed on moist Whatman #1 (9cm) filter paper in petri-plates which were sealed with paraffin paper. Plates were incubated at 16, 20, 25, 30, 35, C for 96 hr, in the dark. There were four leaflets per plate, and four plates per treatment. The conidial production by C. arachidicola was determined as described for the detached leaf experiment.

Conidial germination tests of C. arachidicola at 16, 20, 25, 30, and 35 C. were conducted using the depression slide technique (14). Conidia of C. arachidicola used were produced by incubating leaflets of cv. Tamnut 74, with mature lesions, at 25 ° 1 C under continuous light (800 lux) and 100% relative humidity for 96 hr. Conidia were washed from the surface of leaflets with minimal volumes of distilled water. Concentration of conidia in the suspension was determined with a hemacytometer and then adjusted to 20,000 conidia/ml. Two drops were incubated in each well. Slides were read at 6 and 12 hr after incubation.

RESULTS

Significant (P = .05) differences occured among genotypes for mean number of total lesions per leaflet, mature lesions per leaflet, and rate of lesion increase as estimated by the four largest lesions per leaflet (Table I). The total number of lesions was counted with the leaves held up against a lighted background. Lesions with one or more stroma were considered mature. There was no difference in number of mature lesions between Tamnut 74, Comet, Florunner, and P.I. 109839 (Table 1). There

was no difference in total number of lesions per leaflet among Tamnut 74, Comet, Florunner, P.I. 109830 and P.I. 338280. No Stroma were formed on C. arachidicola lesions in the wild genotypes 21 days after inoculation. However, there was a difference in lesion diameter between Tamnut 74 and Comet compared to P.I. 109830. The difference between Tamnut 74 and Florunner was not significant, but the rate of lesion increase in Comet was higher than in Florunner. Lesions coalesced in Tamnut 74, Comet, and Florunner, however, measurement of the lesion diameter were taken from distinct lesions. Examination of the lesions under the dissecting microscope after 96 hr incubation at 100% RH, revealed the presence of conidiophores but very few conidia on many of the lesions in all genotypes.

There was a significantly (P = .05) higher number of conidia per mm^2 of lesion produced on Tamnut 74 and Comet than on FLorunner and P.I. 109839. There was no difference in number of conidia per mm^2 of lesion between P.I. 109830 and the wild genotypes of hybrides.

The necrotic lesion area was significant (P = .05) among genotypes and ranged from 0.1 to 64.1 mm^2 per leaflet. C. arachidicola inoculation produced minute lesions in the wild genotypes and hybrids. No conidia were recovred from such lesions, however, conidia were recovered from two deteriorating leaflets of P.I. 338280 (409 conidia/mm^2 of lesion). No sporulation was observed on lesions surrounded by green tissue on P.I. 338280. This phenomenon was also reported by Pyzner (11). Examination of leaves of P.I. 338280 and M213 inoculated at the same time as the regular experiment, indicated sporulation of C. arachidicola on lesions surrounded by healthy or chlorotic tissue 32 to 35 days after inoculation, suggesting a prolonged period for lesion maturation for these genotypes.

Multiple linear regression analysis comparing genotype effect was performed on Tamnut, Comet and Florunner using the number of conidia produced per leaflet as predictor variable, and conidia per mm^2 of lesion, total lesions per leaflet, mature lesions per leaflet and necrotic lesion area as four independent variables. The model was significant (R^2 = 0.956, P = .01). There was a genotypic effect on conidia/mm^2 and total lesions, however, a separate coefficient was not required for mature lesions and necrotic lesion area. P.I. 109839 was not included in the model because no conidia were recovered from many leaflets. The regression equations for the three genotypes, estimating the conidia/leaflet is given in Table 2.

Conidial viability was determined by counting the percent germination of C. arachidicola conidia recovered from Tamnut 74, Comet, Florunner, P.I. 109830, and P.I. 338280. There was significantly (P = .01) lower germination for P.I. 338280 compared to Tamnut 74, Comet, Florunner and P.I. 109830 (Table 3). There was no difference in germination of C. arachidicola conidia produced on Tamnut 74, Comet, Florunner, and P.I. 109839.

Pathogenicity tests for conidia of C. arachidicola obtained from different peanut genotypes were conducted on detached leaves of Tamnut 74 following the detached leaf culture technique (9). Conidial suspensions washed from leaflets in each replicate were mixed together providing three conidial suspensions per genotype (inoculum source). No attempt was made to adjust conidial concentration. The inoculum from each replicate was applied on leaflet surfaces with a DeVilbiss No. 15 atomizer. Twelve shoots were inoculated for each inoculum source, representing four shoots per replicate source. All shoots showed C. arachidicola lesions 9 to 10 days after inoculation (Table 4), and inoculum with higher conidial concentration

generally produced the higher number of lesions. All isolates that produced lesions on Tamnut 74 were considered pathogenic.

The effect of temperature on conidial production and germination was studied on Tamnut 74. There was a significant (P = .01) difference in conidia produced per mm^2 of necrotic lesion at different temperatures. The highest number was obtained at 30 C (532 conidia/mm^2 of lesion) and the least conidia produced at 35 C (55 conidia/mm^2 of lesion) as shown in Fig. I. There was no significant difference in conidia per mm^2 of lesion between 16, 20, and 35 C.

The percent germination of conidia of C. arachidicola was also highly significant (P = .01) among temperature conditions. The highest germination was recorded at 25 and 30 C (Fig. 2) at both 6 and 12 hr after incubation. Germination was greatly reduced at 35 C. There was no difference in germination between 6 and 12 hr of incubation at 25, 30, and 35 C, however, the difference at 15 and 20 C was significant (P = .05).

Temperature also affected the length of conidia, which was significantly (P = .05) shorter at 35 C with a mean length of 87 µm. There was no difference in length of conidia among the other temperatures, where a mean length of 111, 92, 92 and 95 µm was recorded at 16, 20, 25 and 30 C respectively.

The effect of light on conidial production and length of conidia of the C. arachidicola isolate used was also examined. Tamnut 74 leaflets with distinct Cercospora lesions were incubated in petri-dish moist chambers for 96 hr. Eight plates containing four leaflets each were exposed to continuous light (800 lux) provided by 40 w Cool White Econo-o-watt florescent, at 25 ± 1 C. Another eight dishes were wrapped in aluminum foil. There was no difference in sporulation between dark and light, however, there was a significant (P = .001) difference in length of conidia

between light and dark. The average length of conidia was 168 and 98 um under light and dark respectively. Length of conidia ranged from 90 to 320 um under light and 50 to 150 um in the dark.

DISSCUSSION

The lack of significance on the number of mature lesions on Tamnut 74, Comet, Florunner and P.I. 109839 may have contributed to the generally low number of conidia produced per mm^2 of lesion. Temperature recorded within the chambers dropped to 20 C night time lows and 26 C day time highs during the third week after inoculation. This may have resulted in reduced rate of lesion increase and lesion maturity. The low conidia per mm^2 recorded on P.I. 109839 compared to Tamnut 74 and Comet may indicate a stronger effect of low temperatures on P.I. 109839 compared to Tamnut 74 and Comet. Results obtained in earlier experiments showed P.I. 109839 (conidia/mm^2) equal to Tamnut 74 and Comet. The number of conidia produced per leaflet on P.I. 109839 was however significantly ($P = .05$) lower than on Tamnut 74 or Comet, and compares with previous results.

P.I. 338280 is one of the parents of M143 [Chico X (P.I. 338280 X P.I. 276235)], and lesions of C. arachidicola on it produced conidia 31 to 35 days after inoculation except when conidia were produced on deteriorating lesions. This genotype is cultivated from seed and may be useful in a breeding program to impart genes for long latent periods. C. arachidicola lesions on M143 did not sporulate even after 35 days after inoculation, which may be due to the presence of resistant genes from P.I. 276235. Chico is as susceptible to C. arachidicola as Tamnut 74 (8).

Profuse sporulation observed on lesions of deteriorating leaflets of P.I. 338280 may suggest a stronger saprophytic nature of C. arachidicola than previously suspected. This phenomenon needs further

investigation.

The lower temperatures retarded conidial production and germination. Lower temperature retard colonization and consequently should influence the rate at which lesion maturity is achieved.

Substantial conidial production is dependent upon higher temperatures (Fig. 1). The fact that infections can take place at lower temperatures (8) is of little importance if existing lesion fail to provide the necessary inoculum. However, since light is not important for sporulaiton of C. arachidicola high minimal night temperatures with high dew periods may increase the sporulating potential of C. arachidicola.

Although there was a significant difference in germination of conidia of C. arachidicola recovered from different peanut genotypes, the germination was quite high (95.6%) on the genotype (P.I. 338280) with the lowest germination. This difference may not contribute much to a reduction of inoculum. Also more conidia may have germinated during a longer period of incubation.

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TABLE 1. Mean number of total lesions, mature lesions, mean lesion diameter, and mean number of conidia produced per mm^2 of necrotic lesion 21 days after inoculation of peanut genotypes with Cercospora arachidicola

Genotype	Parameters[1/]			
	total lesions per leaflet	mature lesions per leaflet	lesion diameter mm^2	conidia per mm^2 of lesion
Tamnut	27.3 ab	4.3 a	2.1 ab	154.4 a
Comet	30.3 a	5.3 a	2.4 a	134.5 a
Florunner	37.3 a	5.3 a	1.8 b	49.8 b
P.I. 109839	20.5 abc	3.9 a	1.4 c	37.2 bc
P.I. 276235	8.9 cd	0 b	0.4 d	0 c
P.I. 276233	10.8 bcd	0 b	0.2 d	0 c
M143[2/]	1.5 d	0 b	0.3 d	0 c
P.I. 338280	31.6 a	0 b	0.4 d	0 c
M213[3/]	4.8 cd	0 b	0.5 d	0 c

[1/] Mean within columns followed by the same letters are not significantly different (P = .05) according to Duncan's multiple range test.

[2/] A hybrid of P.I. 338280 X P.I. 276235

[3/] A hybrid of Chico X (P.I. 338280 X P.I. 276235)

TABLE 2. Regression equations for estimating the number of conidia of Cercospora arachidicola produced per leaflet on peanut genotypes, following a general linear models procedure comparing genotype effects

Genotype	Regression equation[1/]
Tamnut	$Y = -8579.56 + 41.14X_1 + 208.38X_2 + 350.96X_3 + 68.11X_4$
Comet	$Y = -3605.33 + 60.69X_1 - 81.51X_2 + 350.69X_3 + 68.11X_4$
Florunner	$Y = -4257.54 + 90.88X_1 - 18.72X_2 + 350.69X_3 + 68.11X_4$

[1/] Regression model with 25 d.f., $R^2 = 0.96$, C. V. 29.98
Y = Conidia/leaflet
X_1 = Conidia/mm^2 of lesion
X_2 = Total lesions/leaflet
X_3 = Number of mature lesions/leaflet
X_4 = Necrotic lesion area (mm^2)/leaflet

TABLE 3. Germination of Cercospora arachidicola conidia produced on various peanut genotypes, and incubated in slide depression wells for 24 hr under continuous light at 25 ± 1 C.

Conidial Source	Replicate					Mean
	1	2	3	4	5	
Tamnut 74	100.0	99.5	96.0	98.0	96.5	98.0
Comet	100.0	100.0	99.5	100.0	99.0	99.7
Florunner	98.5	97.0	99.0	99.5	98.0	98.4
P.I. 109839	98.5	98.5	100.0	97.0	99.0	98.6
P.I. 338280	94.0	96.0	94.5	97.0	96.0	95.5
LSD (.01)						2.1

1/ Mean of two slide depression wells, 100 conidia were read per depression well.

TABLE 4. Pathogenicity reaction of Cercospora arachidicola on a susceptible peanut c.v. Tamnut 74, 21 days after inoculation with conidia isolated from different peanut genotypes.

Inoculum source			Number of lesions produced per leaflet [1/]
Genotype	Replicate	Conidia per ml	
Tamnut 74	1	16500	8.5
	2	20750	7.4
	3	13500	8.0
Comet	1	13000	1.6
	2	14250	4.6
	3	24250	11.7
Florunner	1	1000	5.4
	2	1000	2.0
	3	25250	16.7
PI 109839	1	<1000	1.2
	2	3250	2.3
	3	3250	2.2

1/ Mean of 16 leaflets. All isolates were considered pathogenic.

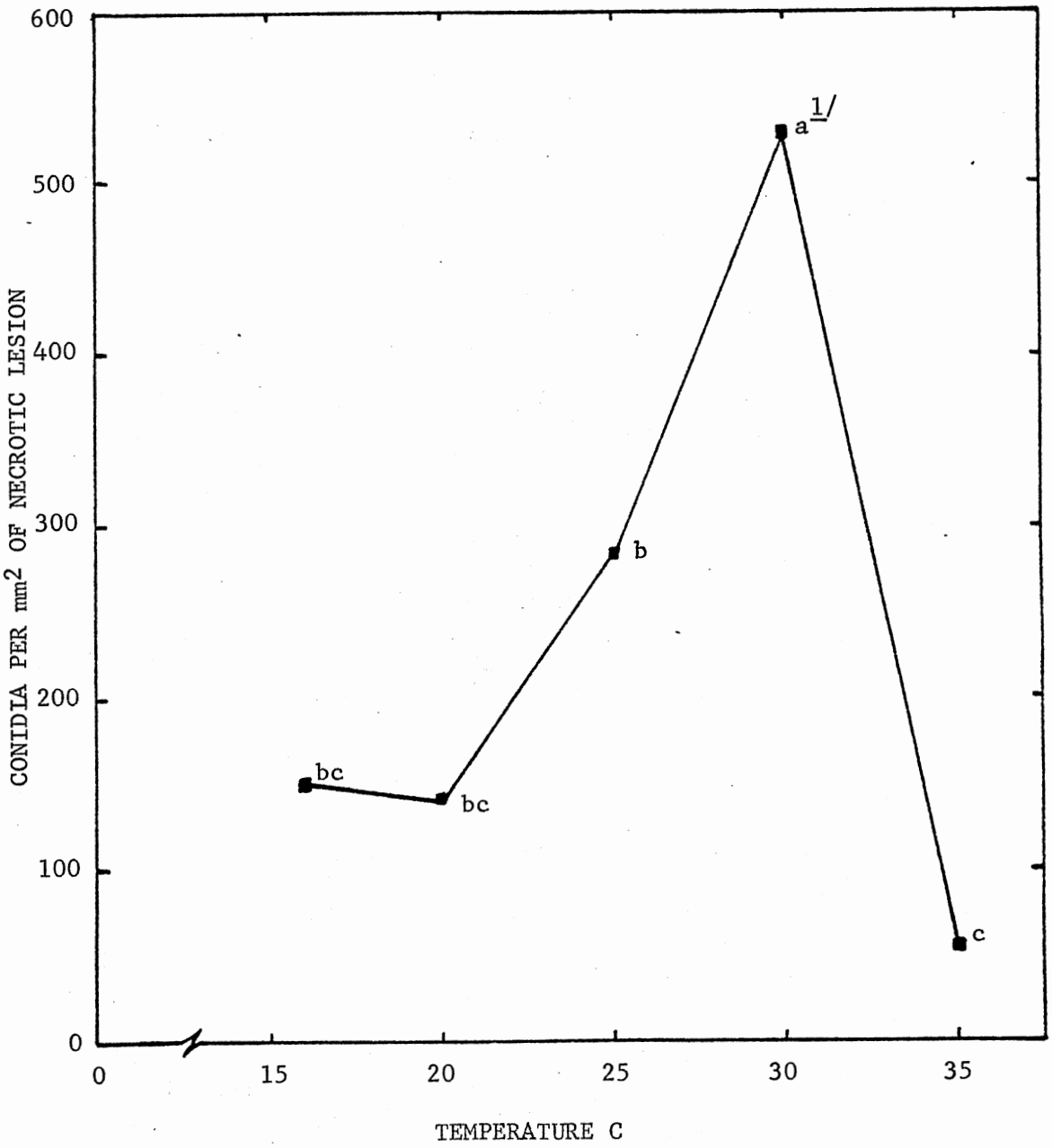

Fig. 1. Effect of temperature on the mean number of conidia of Cercospora arachidicola produced per mm^2 of necrotic lesion on c.v. Tamnut 74.
1/ Means followed by the same letter are not significantly different (P = .05) using Duncan's new multiple range test.

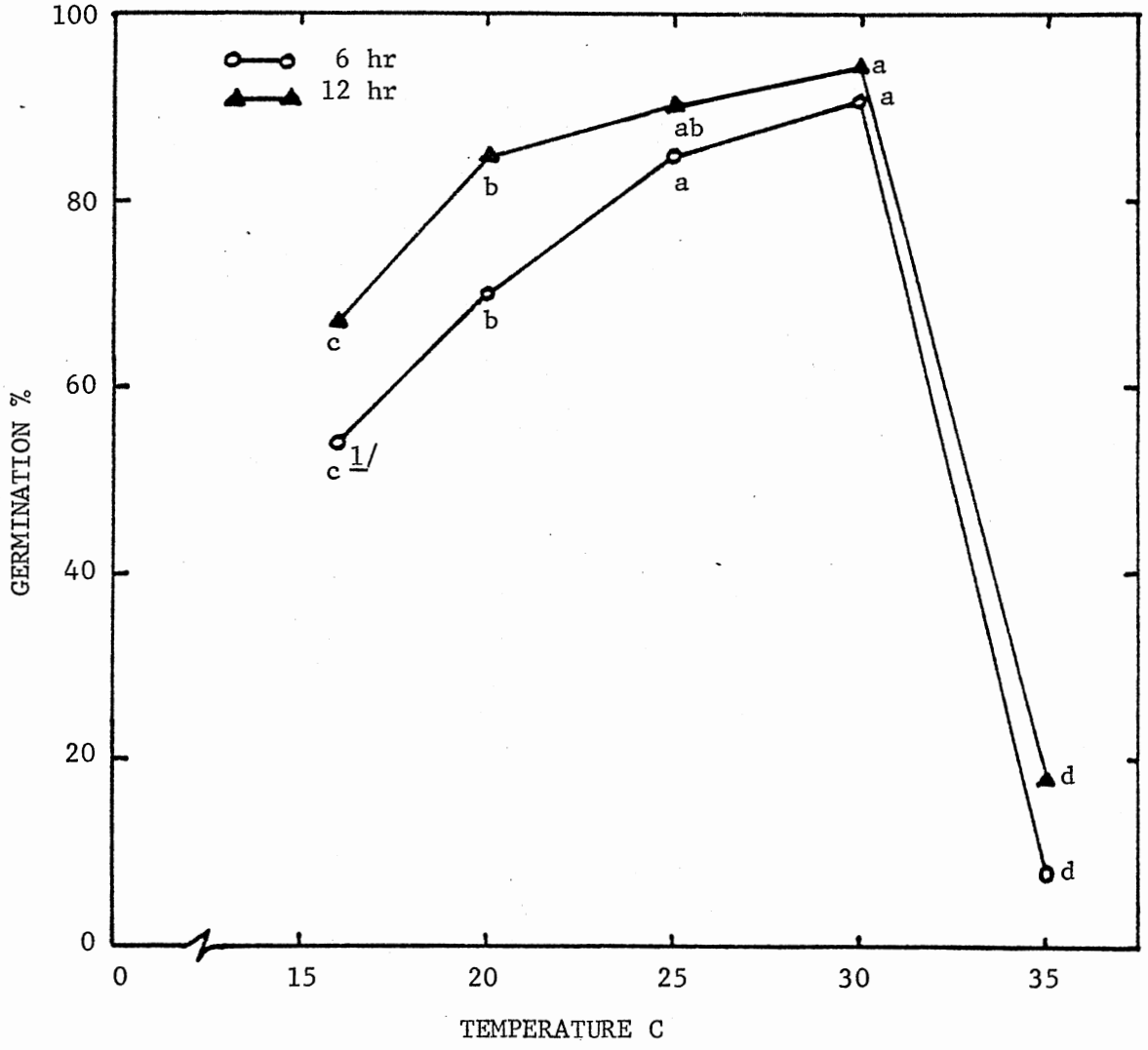

Fig. 2. Effect of temperature on conidial germination of Cercospora arachidicola incubated in the dark for 6 and 12 hr.

1/ Means within the same period followed by the same letter are not significantly different (P = .05), using Duncan's new multiple range test.

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