ENVIRONMENTAL FACTORS INFLUENCING CONIDIAL RELEASE AND SPORULATION OF PERONOSPORA MANSHURICA

A Thesis

by

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(Chairman of Committee)

(Member)

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(Head of Department)

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ABSTRACT

Environmental Factors Influencing Conidial Release and Sporulation of Peronospora manshurica. (December 1980)
David Brian Madden, B.S., Texas A&M University
Chairman of Advisory Committee: Dr. S. D. Lyda

The liberation of Peronospora manshurica (Naoum.) Syd. ex Gaum. conidia was studied under natural field conditions with the use of spore traps and hygrothermographs. Conidial liberation began approximately 0700 to 0800 hr with the maximum conidial release occurring between 1000 to 1200 hr Conidial liberation closely corresponded to a decrease in relative humidity and an increase in morning winds. The drop in relative humidity is thought to cause a drying and twisting of the conidiophore which caused ejection of the conidia. The nighttime temperature ranged between 15-20 C which is well within the optimum temperature range (10-25 C) for sporulation of P. manshurica. For this reason, it seems that the nocturnal relative humidity is the limiting factor in sporulation and, thus, the amount of conidial liberation. When number of hours of optimum humidity versus number of conidia released was analyzed statistically, the correlation coefficient was approximately 0.12 which suggested a very poor relationship between humidity and sporulation. Cruickshank (5) showed that it is not a simple relationship which exist between optimum humidity and sporulation. It only takes

optimum humidity during a short critical time period to produce abundant sporulation. Sporulation studies were conducted to determine if P. manshurica would behave in the same manner as P. tabacina, however all attempts to do so have failed for several reasons.

ACKNOWLEDGEMENTS

I am especially grateful to Dr. S. D. Lyda, my Committee Chairman, for his assistance and encouragement throughout the course of this study. I am also grateful to the members of my committe, Dr. D. H. Smith, Dr. D. J. Mueller and R. A. Taber. I also express my thanks to Philip C. Lyons, Charles M. Rush, Karen S. Arthur, Pamela Calvin, Hang Lua and Robin Shannon, fellow students, for assistance and encouragement during this study.

I would like to dedicate this work to Linda Diane Madden, Mr. and Mrs. Frank H. Madden and Mr. and Mrs. Richard J. Bozeman. Without their support, I would not have completed this work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The soybean, Glycine max (L.) Merrill, is native to eastern Asia, and has hundreds of food, feed and industrial uses. The crop is currently grown in many regions of the world and is a primary source of vegetable oil and protein. Forty percent of the total supply of edible vegetable oil comes from soybeans. In the United States, over 90% of the soybean meal, a by-product of the oil-extracting processes, is used in livestock and poultry feeds. Soybean products are very important components of low-cost, nutritional, high protein foods for human consumption (24).

In 1977, approximately 6.0×10^7 acres of soybeans were planted in the United States (26). This accounts for about 54% of the world soybean acreage; however, the yield on this acreage is 64% of the world soybean production.

The increased soybean acreage and production has been accompanied by a concomitant increase in the number and severity of soybean diseases. In 1977, about 25% of the soybean crop in the southern United States was lost to disease. Downy mildew caused by Peronospora manshurica (Naoum.) Syd. ex Gaum. was responsible for about 1% of the total amount of disease losses or approximately 1.3×10^6 bushels (bu) (29). In 1977, 8.0×10^5 acres of soybeans

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were planted in Texas (27). Approximately 12% of this soybean acreage was lost to disease. Downy mildew was responsible for about 1.6% of the total amount of disease losses or 4.3×10^4 bu (29).

While downy mildew is normally a minor disease problem on soybean, it can become severe. The disease can cause as much as 8 to 10% disease loss under optimum environmental conditions (24). The influence of some of these environmental conditions on the disease and spread of the pathogen is unknown. The objectives of this study were to determine:

a) The maximum period of conidial release by the pathogen.

b) The effect of relative humidity on conidial release.

c) Efficacy of four fungicides against P. manshurica.

LITERATURE REVIEW

Downy mildew of soybean was first reported in 1908 from Kashmir (24). In 1912, Naoumoff (19) identified the causal organism of soybean downy mildew as Peronospora trifoliorum de Bary var. *manshurica* Naoumoff while in the Russian Far East. Miura (18) reported a fungus which caused downy mildew of soybean in Manchuria in 1921. The causal fungus was Peronospora trifoliorum var. manshurica. In 1922, Haskell and Wood (in Hildebrand and Koch, 1951) reported the first occurrence of the disease on soybean in the United States (10). In 1923, Lehman and Wolf (14,15) reported the disease in North Carolina; however, they believed that it was an undescribed species of Peronospora. They named it Peronospora sojae Lehman and Wolf. In the same year Gaumann raised P. trifoliorum var. manshurica to the rank of species, and thus, it became P. manshurica (10, 30). In 1926, Wolf and Lehman (30), after several comparisons with isolates from Manchuria, concluded that the downy mildew organism in North Carolina was identical to the P. trifoliorum var. manshurica. It was also morphologically identical to Gaumann's species manshurica; thus, the causal organism of soybean downy mildew is now P. manshurica (Naoum.) Syd. ex Gaum. and P. sojae Lehman and Wolf became a synonym (30). Since its discovery in Kashmir and Manchuria, this disease has become worldwide in distribution (4, 7, 10, 24).

The downy mildew fungus attacks primarily leaves, but it frequently attacks pods. Abramoff (1) reported that "Downy mildew (Peronospora manshurica) may either produce localized infection, in which case the damage is insignificant, or systemic causing a noticeable stunting of all the aerial organs of the host with a consequent considerable reduction in yield." Abramoff, however, did not speculate on the nature of the systemic infection. Thus, soybean downy mildew was considered to be a foliar disease until 1942. In that year, Johnson and Lefebvre (12) reported the occurrence of oospores on the seed coat; however, they made no reference to the systemic nature of the disease. In 1945, Hildebrand and Koch (in Hildebrand and Koch, 1951) and in 1946, Jones and Torrie (13) demonstrated that the oospore-encrusted seed gave rise to systemically-infected soybean.

The effect of P. manshurica on soybean is variable. Some systemically-infected seedlings develop into stunted plants which do not survive the entire growing season; however, they do produce enormous numbers of conidia. Other systemically-infected seedlings survive the growing season, but exhibit a stunted, spindly type of growth. These plants produce seed, but the seed is reduced in both number and size.

The most conspicuous symptom in the systemic infection phase appears on the first true leaves as a clearly defined

lighter green area. The lighter green area is seen on the lamina near the point of its juncture with the petiole. Infection progresses along the midvein in a serrated pattern or fans out from the point of infection. The disease next progresses to the first trifoliate leaves; the symptoms on these leaves are similar to symptoms seen on the first true leaves. Finally, the entire plant shows the systemic infection symptoms (10).

Within a week to 10 days after the expression of symptoms, sporulation occurs on the underside of the lighter green areas on the leaves. The conidia serve as secondary inocula; therefore, the initial spread is from systemically-infected plants which serve as foci for spread of the pathogen to other healthy plants (4, 10, 16).

The spread of the pathogen through the field is influenced by several environmental factors. The effect of temperature on sporulation and disease development is known, but the role of relative humidity is not understood (17, 24). Likewise, the period of maximum conidial release and the roles that temperature and relative humidity play in spore release are not known.

After conidial release from a systemically-infected plant, they are dispersed by the wind. Infection by these spores will induce the local lesion phase of the disease. The symptoms of this phase are different from the symptoms of the systemic phase. Local lesion symptoms are character-

ized by the occurrence of indefinite chlorotic flecks on the upper surface. As the flecks enlarge, they become dark-brown to reddish-brown with indefinite yellow halos. Abundant conidia are produced on the underside of the diseased leaf (4, 7, 10, 24). Unlike the systemic infection, the local lesion does not significantly reduce yield, unless it is a severe infection (1, 10).

During seed formation, if the pods are infected, the seed may become infected and, thus, become encrusted with oospores. This occurs when the endocarpic tissue is replaced by fungal mycelium and oospores which adhere to the seeds when the pods open. This results in oospore-encrusted seeds. Apparently, the fungus does not penetrate the seed coat and provide inocula for infections which result in systemically-infected plants.

When the plants start to senesce, the fungus forms oospores. In the systemically-infected plants, the oospores are found in almost all plant tissues. In the local lesion infected plants, the oospores are found in the leaf and pod tissues. Thus, oospores, which are the overwintering stage, can survive on the seed coat or in plant debris (4, 7, 10, 24).

The control of downy mildew is achieved by two methods. First, Hildebrand and Koch (10) demonstrated that seed treatment fungicides reduced the incidence of systemic infection. The second and most widely used method is disease resistance.

Currently, there are 39 physiological races of P. manshurica and various varieties are susceptible to various physiological races (4, 8, 9).

MATERIALS AND METHODS

Spore release (field study). The maximum period of conidial release was investigated. The study was conducted at the Upland Farms, Texas A&M University, College Station, TX. The plot was planted with Bragg, ie, a highly susceptible soybean variety. The plot consisted of eight 110m rows about 96cm apart, planted at the rate of eight seeds per 30.5cm of row. The prevailing winds were from the south-southeast and the rows were planted east to west. After natural infection had occurred in the plot, conidia were collected from 20 August, 1979 to 28 September, 1979. Four 7-day recording hygrothermographs recorded the temperature and relative humidity (RH). Other climatic data were provided by Texas Farm Services (Table 1) located 0.7km southwest of the plot site. The hygrothermographs were enclosed in weather instrument shelters at an elevation of 0.5m (Figs. 1A and 1B) (28). The conidial samples were taken with four spore samplers (Fig. 2A). They were trapped on 24 petroleum jelly-coated microscope slides (Fig. 2B) (21). The slides were examined at 100x and the conidial numbers recorded at 2-hr intervals. The samples were collected continuously during the week, but they were not collected on the weekends during the period of the study. Since the spore samplers were powered by 12V wet batteries, the air flow could not be held constant. The air flow was measured at 2-hr

TABLE 1. Climatic data collected by Texas Farm Services during the study period of 20 August to 28 September, 1979.

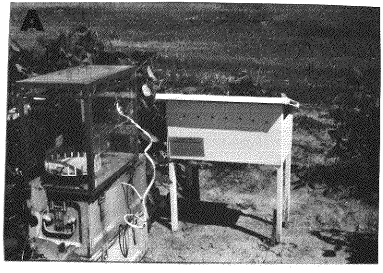
Date	Wind direction	Atmosphere [a]	Precipitation	Wind speed [b]	Dew
Aug 20	SW	P1.C		M	+
21	SE	P1.C		M	+
22	SE	P1.C		M	+
23	SE	P1.C	1.8	M	+
24	SE	C1.		M	+
25	SE	C1.		M	+
26	SE	C1.		M	+
27	SE	C1.		M	+
28	SW	P1.C		M	+
29	SE	P1.C		M	+
30	SE	Co.	0.5	M	+
31	SE	P1.C		M	+
Sept 1	SE	P1.C		M	+
2	SE	P1.C		M	+
3	SE	C1.		M	+
4	SE	P1.C		M	+
5	SE	P1.C		M	+
6	SE	P1.C		M	+
7	SE	P1.C		M	+
8	SE	P1.C		M	+
9	SE	P1.C		M	+
10	SE	P1.C		M	+
11	SE	P1.C		M	+
12	N	P1.C		M	-
13	N	P1.C		M	-
14	N	C1.		M	-
15	N	C1.		M	-
16	N	C1.-P1.C.		M	+
17	N	C1.-P1.C.		M	+
18	N	Co.	3.0	M	-
19	N	Co.	2.8	M	-
20	N	Co.	2.0	M	-
21	SE	C1.		M	-
22	SE	C1.		M	+
23	SE	C1.		M	+
24	SE	C1.		M	+
25	SE	C1.		M	+
26	SE	C1.		M	+
27	SE	C1.		M	+
28	SE	C1.		M	+

[a] Atmospheric condition as defined by the National Weather Service P1.C (Partly Cloudy); C1. (Clear); Co. (Cloudy).

[b] Wind speed as defined by the National Weather Service. M (Moderate).

Fig. 1A. Spore sampler and weather instrument shelter.

Fig. 1B. Hygrothermograph enclosed in a weather instrument shelter.


A

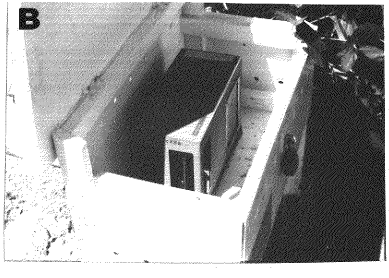
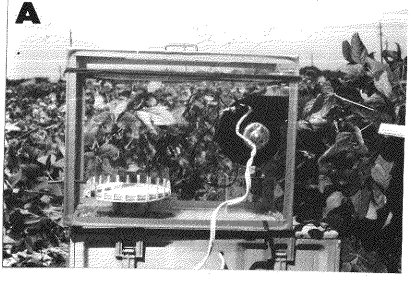
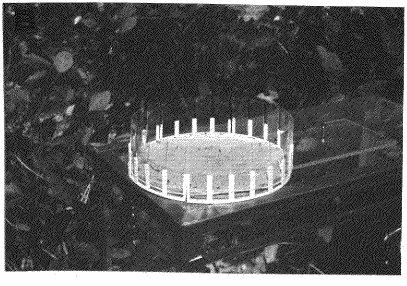

B

Fig. 2A. Spore sampler used in the conidial release study. The 12V batteries are housed in the metal boxes.

Fig. 2B. The 24 petroleum jelly-coated microscope slides held on a rotating wooden disc.

A





intervals for 24 hrs with a hot wire anemometer. A calibration curve was prepared (Fig. 3 and Table 2) and the numbers of conidia per hr were adjusted accordingly (Appendix).

Spore release (laboratory study). A study was conducted to determine the influence of RH on the spore release of P. mansnurica in the laboratory. Systemically-infected plants were obtained from oospore-encrusted seed by a method described by Grabe and Dunleavy (9). The resultant seedlings were observed for systemic infection ranging from 10 to 20%. Conidia were induced to form on the systemically-infected plants by spraying the plants with water, placing a plastic bag over the potted plants and maintaining the temperature between 20 to 22C for 12 hrs. The suspension of conidia was sprayed on Bragg seedlings and then placed in a plastic bag for 24 hrs at 20 to 22C (9, 31).

Petri dish chambers having RH ranging from 30 to 100% were used to study the effects of RH on spore release. The petri dish chambers were constructed by lining 90mm-deep dishes with filter paper. The various relative humidities were achieved by using varying ratios of glycerol to distilled water (20). Filter paper moistened with distilled water was used for 100% RH. Downy mildew infected leaves with conidia sporulating on the underside were placed onto a plastic ring. This ring sat on a petroleum jelly-coated microscope slide which was on the bottom of the petri dish

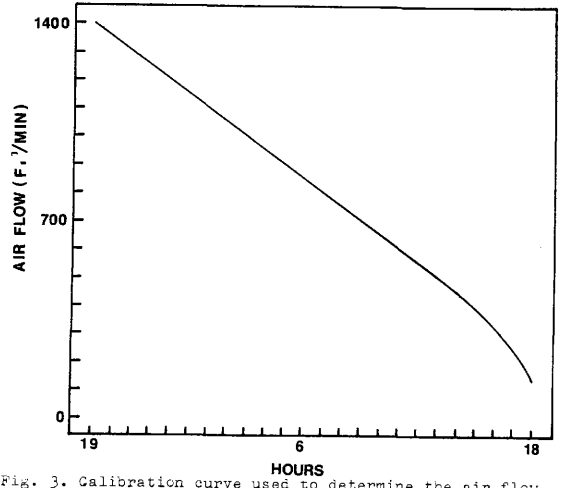


Fig. 3. Calibration curve used to determine the air flow (ft^3/min) for each hour. (Note that the batteries were changed at 1900 hr)

TABLE 2. Air flow and correction factor used to adjust the spore numbers to a standardized air flow rate (1400 ft^3/min).

Hour [a]	Air flow (ft^3/min) [b]	Correction factor [c]
19	1400	1.00
20	1350	1.03
21	1300	1.07
22	1250	1.12
23	1200	1.16
24	1150	1.21
1	1100	1.27
2	1050	1.33
3	1000	1.40
4	950	1.47
5	900	1.55
6	850	1.64
7	800	1.75
8	750	1.86
9	700	2.00
10	650	2.15
11	600	2.33
12	550	2.54
13	500	2.80
14	450	3.11
15	380	3.68
16	330	4.24
17	250	5.60
18	180	7.77

[a] 12V wet batteries were charged at 19 00 hr every day during the study.

[b] Flow rate measured with a hot wire anemometer.

[c] Correction factor calculated by dividing 1400 ft^3/min by the flow rate measured at that hour.

RH chamber. The chamber, which represented the 100% RH chamber, was closed for 5 min, then the leaf was removed and placed in the 90% RH chamber. This procedure was followed until the leaf had been placed in all the chambers from 100% to 30%. The slides were removed and examined at 100x and the number of conidia per unit area was recorded.

Sporulation. A study was conducted to determine the relationship between RH, time and sporulation. In this study, blocks of leaves were set up in RH chambers at 1800, 2000, 2200, 2400, 0100, 0200, 0300 hr. Samples of leaves were removed for measurement of sporulation from each block at 0400, 0500, 0600, 0700 and 0800 hr. This study was conducted at 100, 95 and 90% RH or until no sporulation occurred.

The amount of sporulation was measured by taking a core bore and removing a portion of a downy mildew lesion. The lesion was placed in 1ml 50% ethanol in test tubes. They were shaken for 30 sec and the concentrations of the spore suspension measured with haemocytometer. The spore concentration was taken as a measure of sporulation intensity.

Fungicide Tests. Four fungicides were screened for activity against P. manshurica on four varieties of soybeans. The varieties Bragg, Dowling, Alamo, and V-1 were planted in plots that were 4m long and 4 rows wide (96cm apart).

The plots were sprayed with the experimental fungicides LS 74-783 (2.2kg ai/ha), CGA-48988 (0.14kg ai/ha), the commercial fungicides Top Cop (37.0kg ai/ha) and Manzate 200(2.2kg ai/ha). The fungicides were applied twice on a two-week schedule at the R3 stage of growth (ie, beginning pod set). The variety-fungicide combinations had four replications and four check plots corresponding to each variety. The middle two rows of each plot (equivalent to 0.0008ha) were harvested and yield data were collected. Likewise, 100-seed weight and germination data were obtained.

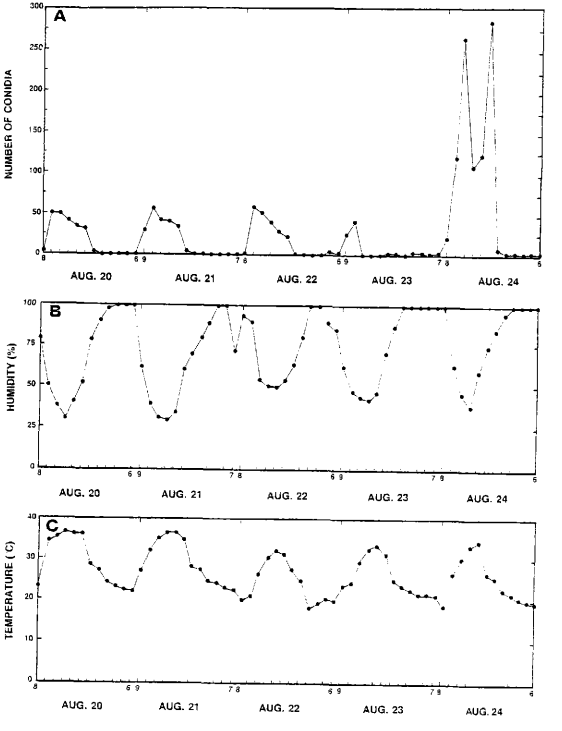
RESULTS

Spore Release (field study). The environmental conditions were recorded on a continuous basis during the entire period of the study. However, spore samples were collected only during a portion of the study. Week 1 corresponds to the dates 20-24 August; Week 2: 27-31 August; Week 3: 3-7 September; Week 4: 10-14 September; Week 5: 17-21 September; and Week 6: 24-28 September.

The actual numbers of conidia collected were corrected with the use of a calibration curve and correction table (Fig. 3 and Table 2). After the corrected conidial numbers were calculated, it was determined that the period of maximum conidial release was approximately 1000 hr to 1200 hr (Figs. A, 4-9). This peak period of conidial release was observed during the entire period of the study with only a few exceptions. On 24 August (Fig. 4A), the peak period was at 1200 hr and 1800 hr and likewise the peak period was 1700 hr on 27 August (Fig. 5A). During the periods of 11-14, 17-19 September, no conidia were recorded (Figs. 7A and 8A).

A Duncan's multiple range test was conducted on all the corrected conidial numbers within each day. The test showed that the conidial numbers recorded during the peak period (1000hr to 1200hr) were significantly higher than the conidial numbers recorded outside the peak period of conidial release (Appendix). In a few cases, this was

Fig. 1. The data recorded at 2 hr intervals from 20 August to 24 August, 1979 at the soybean downy mildew nursery. A date corresponds to a 24 hr period beginning eitner at 0600 or 0900 hr and ending at 0600 or 0700 hr. A. Number of conidia. B. %RH. C. Temperature.


A
NUMBER OF CONIDIA
300
250
200
150
100
50
0
AUG. 20
AUG. 21
AUG. 22
AUG. 23
AUG. 24
B
HUMIDITY (%)
100
75
50
25
0
AUG. 20
AUG. 21
AUG. 22
AUG. 23
AUG. 24
C
TEMPERATURE (C)
40
30
20
10
0
AUG. 20
AUG. 21
AUG. 22
AUG. 23
AUG. 24

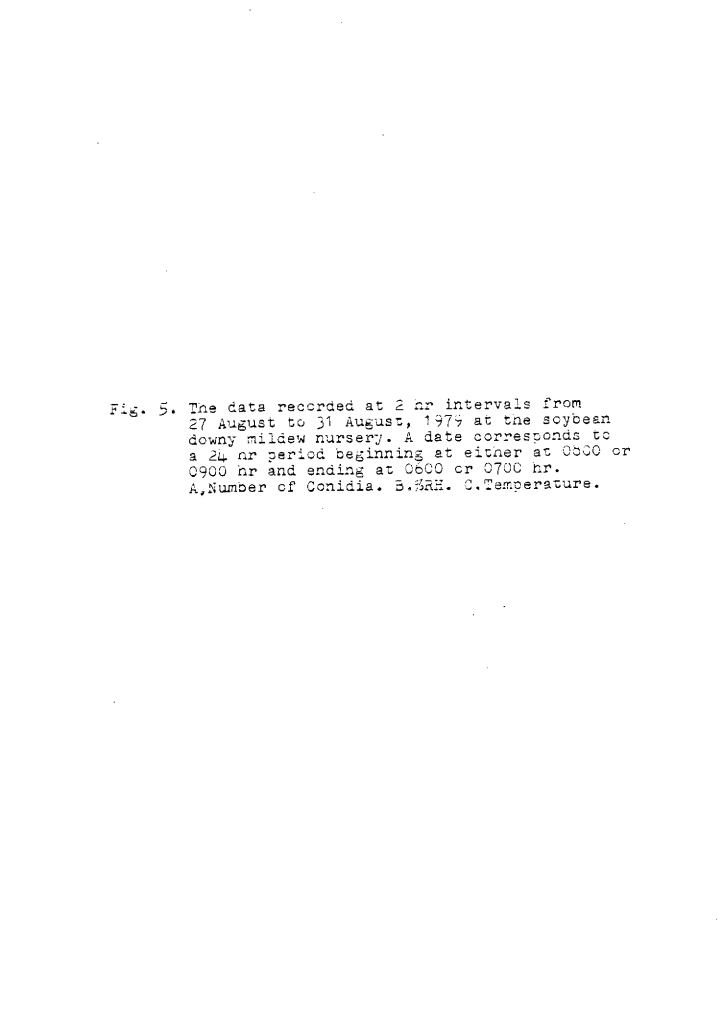


Fig. 5. The data recorded at 2 hr intervals from 27 August to 31 August, 1979 at the soybean downy mildew nursery. A date corresponds to a 24 hr period beginning at either at 0500 or 0900 hr and ending at 0600 or 0700 hr. A,Number of Conidia. B.%RH. C.Temperature.

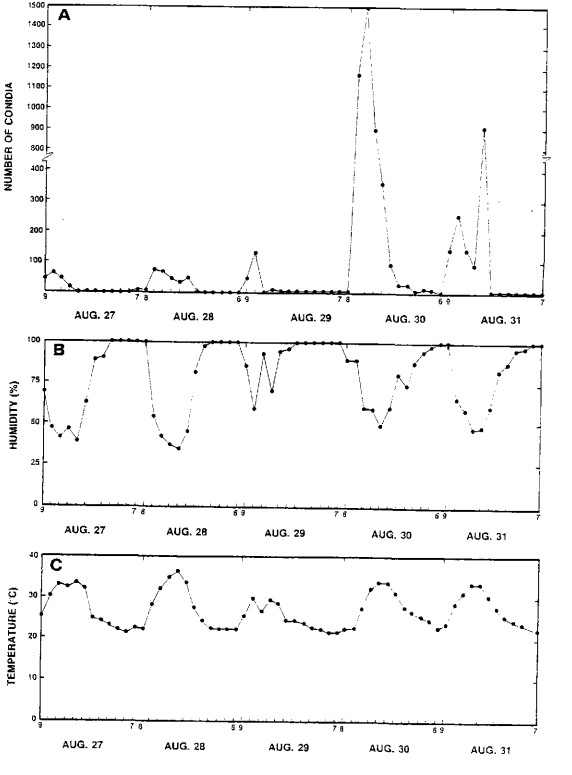

A
NUMBER OF CONIDIA
1500
1400
1300
1200
1100
1000
900
800
400
300
200
100
AUG. 27
AUG. 28
AUG. 29
AUG. 30
AUG. 31
B
HUMIDITY (%)
100
75
50
25
0
AUG. 27
AUG. 28
AUG. 29
AUG. 30
AUG. 31
C
TEMPERATURE (°C)
40
30
20
10
0
AUG. 27
AUG. 28
AUG. 29
AUG. 30
AUG. 31

Fig. 6. The data recorded at 2 hr intervals from 3 September to 7 September, 1979 at the soybean downy mildew nursery. A date corresponds to a 24 hr period beginning either at 0800 or 0900 hr and ending at 0600 or 0700 hr. A.Number of Conidia. B.%RH. C.Temperature.

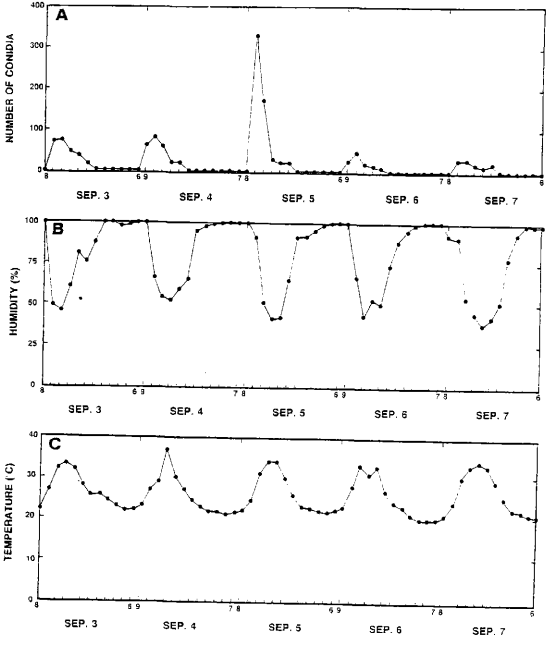

A
NUMBER OF CONIDIA
400
300
200
100
0
8
6 9
7 8
6 9
7 8
6
SEP. 3
SEP. 4
SEP. 5
SEP. 6
SEP. 7
B
HUMIDITY (%)
100
75
50
25
0
8
6 9
7 8
6 9
7 8
6
SEP. 3
SEP. 4
SEP. 5
SEP. 6
SEP. 7
C
TEMPERATURE (C)
40
30
20
10
0
8
5 9
7 8
6 9
7 8
5
SEP. 3
SEP. 4
SEP. 5
SEP. 6
SEP. 7

Fig. 7. The data recorded at 2 hr intervals from 10 September to 14 September, 1979 at the soybean downy mildew nursery. A date corresponds to a 24 hr period beginning either at 0800 or 0900 hr and ending at 0600 or 0700 hr.
A.Number of Conidia. B.%RH. C.Temperature.

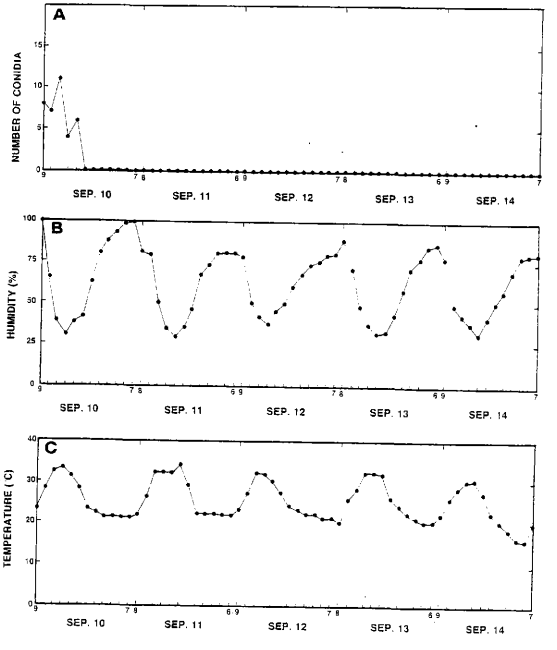

A
NUMBER OF CONIDIA
15
10
5
0
9
7 8
6 9
7 8
6 9
7
SEP. 10
SEP. 11
SEP. 12
SEP. 13
SEP. 14
B
HUMIDITY (%)
100
75
50
25
0
SEP. 10
SEP. 11
SEP. 12
SEP. 13
SEP. 14
C
TEMPERATURE (C)
40
30
20
10
0
SEP. 10
SEP. 11
SEP. 12
SEP. 13
SEP. 14

Fig. 8. The data recorded at 2 hr intervals from 17 September to 21 September, 1979 at the soybean downy mildew nursery. A date corresponds to a 24 hr period beginning either at 0800 or 0900 hr and ending at 0600 or 0700 hr. A,Number of Conidia. B.%RH. C.Temperature.

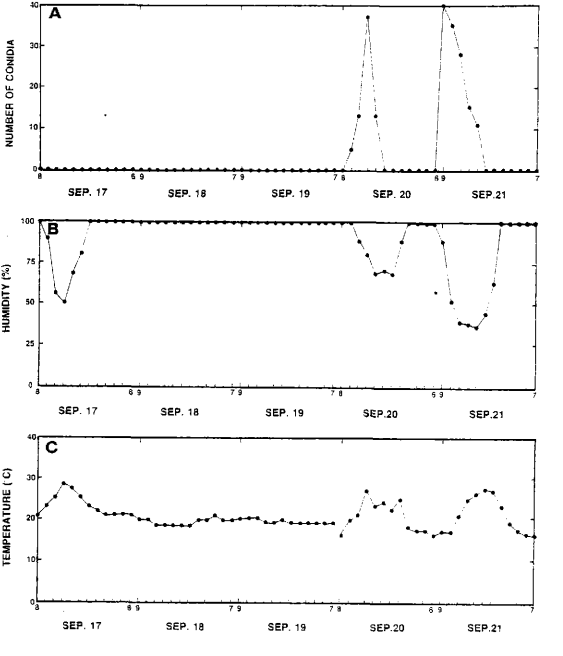
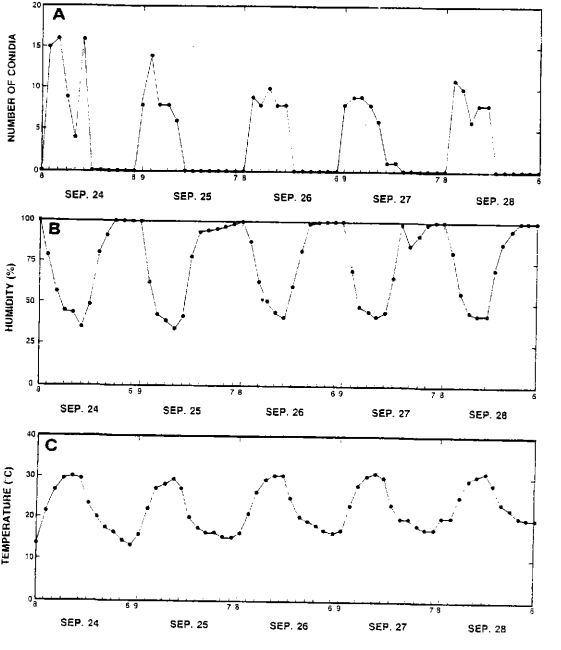

A
NUMBER OF CONIDIA
SEP. 17
SEP. 18
SEP. 19
SEP. 20
SEP.21
B
HUMIDITY (%)
SEP. 17
SEP. 18
SEP. 19
SEP.20
SEP.21
C
TEMPERATURE (C)
SEP. 17
SEP. 18
SEP. 19
SEP.20
SEP.21

Fig. 9. The data recorded at 2 hr intervals from 24 September to 28 September, 1979 at the soybean downy mildew nursery. A date corresponds to a 24 hr period beginning either at 0800 or 0900 hr and ending at 0600 or 0700 hr. A.Number of Conidia. B.%RH. C.Temperature.


A
NUMBER OF CONIDIA
20
15
10
5
0
SEP. 24
SEP. 25
SEP. 26
SEP. 27
SEP. 28
B
HUMIDITY (%)
100
75
50
25
0
SEP. 24
SEP. 25
SEP. 26
SEP. 27
SEP. 28
C
TEMPERATURE (°C)
40
30
20
10
0
SEP. 24
SEP. 25
SEP. 26
SEP. 27
SEP. 28

not observed (ie, 24 and 27 August and 11-14, 17-19 September).

The RH ranged from 100 to 35%. The number of hours in which the RH was 100% ranged from 4-24 hr with an average of 6-12 hr (Figs. B, 4-9). The only exception to this was during September 11-14, when the RH did not exceed 92% (Fig. 7B).

The nocturnal temperatures were within the range necessary for disease development (20-24C) and also within the range required for sporulation (10-25C). On the other hand, the daytime temperatures were above the range required for sporulation and disease development (Figs. C, 4-9).

Additional climatic data were obtained from Texas Farm Services which was 0.7km south of the study area (Table 1). The wind direction was usually from the south except between September 12-20 when the wind direction was from the north. The atmospheric conditions ranged from cloudy to clear. The wind speed was moderate and precipitation was recorded on five days during the study. The presence of dew was determined at 0800hr, but the duration of dew on the leaf surface was not determined. In determining the length of time that dew was present, it was assumed that the difference between air temperature and leaf temperature was zero. Based on this assumption, the number of hours of 100% RH is equivalent to the number of hours that dew was present on the leaf surface of the soybean plant in the soybean downy

mildew nursery.

Spore release and sporulation. Since P. mansnurica is an obligate parasite, the organism must be maintained on the soybean plant. In order to conduct both spore release and sporulation studies, soybean seedlings must be infected with the local lesion phase of soybean downy mildew. When the four methods described by Grabe and Dunleavy (9) to obtain systemically-infected soybean were attempted, they failed to produce systemically-infected soybeans. Each method was attempted several different times, but as of this date, they have failed to produce any signs of systemic infection.

Another method was attempted which involved the use of soybeans which expresses local lesion symptoms. These soybeans were brought in from Beaumont and when placed in 100% RH for 24 hr at 21C, they produced an abundant number of conidia. Approximately 200 to 250 soybean seedlings were inoculated by the method described above. After 10 days, about 80% of the inoculated soybean exhibited symptoms of local lesion infection. However, when the soybean leaves were used in the sporulation study, they failed to sporulate at any of the time blocks or relative humidities. With the failure of this particular experiment, we were forced to rely on the Grabe and Dunleavy methods for the production of any further inoculum. We feel that since we have had no

success thus far with these methods, we would be forced to wait another growing season until the disease occurs naturally in the field. Even with the occurrence, we are not guaranteed that the fungus will sporulate under laboratory conditions. The reasons for the lack of success with the Grabe and Dunleavy methods and the non-sporulation of the fungus are not known at this time.

Fungicide Tests. The factors used to evaluate four fungicides for activity against P. manshurica were yield (kg/ha), 100-seed weight, and percent germination. When the yield means were evaluated, Dowling and V-1 showed no significant difference among the treatments. However, Alamo and Bragg were significantly different, but LS 74-783 exhibited the lowest mean (Table 3). The greatest variation was exhibited in 100-seed weight. There was no significant difference observed among treatments on Alamo, but Bragg, Dowling and V-1 showed differences among the different treatments. Dowling was the only variety in which the check exhibited the smallest 100-seed weight mean (Table 3). When percent germination was evaluated, only Alamo showed any difference among treatments (Table 3).

TABLE 3. Yield, 100-seed weight, and germination data from the fungicide screening program conducted at Upland Farm, Texas A&M University on the soybean downy mildew disease nursery.

Variety	Fungicide	Yield (Kg/ha)	100 Seed weight (g)	Germination
Alamo				
	CGA 48988	676.91 ab [x]	10.91 a	95.5 a
	LS 74-783	403.95 b	11.62 a	80.8 a
	Manzate 200	811.73 ab	11.45 a	80.0 ab
	Top Cop [y]	973.89 a	12.21 a	78.8 ab
	Check	523.17 ab	11.31 a	71.1 b
Bragg				
	CGA 48988 [y]	998.71 ab	15.40 a	95.0 a
	LS 74-783 [y]	762.25 b	14.38 b	87.5 a
	Manzate 200	1301.65 a	14.37 b	95.5 a
	Top Cop [y]	854.04 ab	14.89 ab	88.3 a
	Check [y]	1106.70 ab	15.13 a	93.0 a
Dowling				
	CGA 48988	1395.90 a	15.44 ab	90.0 a
	LS 74-783	842.72 a	16.06 a	82.2 a
	Manzate 200	1010.87 a	15.36 ab	81.1 a
	Top Cop	858.96 a	16.11 a	88.8 a
	Check [y]	1054.98 a	14.72 b	90.8 a
V-1				
	CGA 48988	912.10 a	10.29 c	94.4 a
	LS 74-783	779.84 a	10.06 c	91.1 a
	Manzate 200	1074.74 a	11.48 a	95.5 a
	Top Cop	938.23 a	10.58 bc	93.3 a
	Check	791.44 a	10.97 ab	94.4 a

[x] Means in each column followed by the same letter do not differ significantly (P=0.05) by Duncan's multiple range test. Duncan's multiple range test was conducted only on treatments within each variety.

[y] Means based on four samples.

DISCUSSION

Spore release and sporulation. It has been noted that the peak period of conidial release in most cases closely corresponds to the time period 1000 hr to 1200 hr (Figs. A, 4-9). This release period coincides with a decrease in the relative humidity (RH) which occurs at approximately the same time (Figs. B, 4-9). The decrease in the RH triggers the primary mechanism responsible for the release of the conidia. The decrease in RH causes a drying and twisting of the conidiophores. This twisting along the longitudinal axis of the conidiophores facilitates the release of the conidia (5, 6, 22, 23, 25). Another mechanism which plays a less important role in the release of conidia is the dislodging action of wind and mechanical shock (6). Once released, the conidia become air-borne and the conidia of some downy mildew organisms are dispersed for considerable distances (11).

It was observed that conidial release occurred past the peak period (Figs. A, 4-9). This possibly is due to differential drying of the crop canopy, ie, the outer portion dries before the inner portion. This differential drying could be the mechanism responsible for the extended period of conidial liberation.

Whether the conidia can cause infection is not known; however, several investigators have shown that the conidia

of several downy mildew organisms can survive longer periods under adverse environmental conditions than was once thought (2, 3, 22, 25). Pederson (22) showed that as many as 15% of the conidia released during the morning hours by P. manshurica were able to survive the day and caused infection the following night. Infection occurred only when favorable environmental conditions were present, ie, high humidity and suitable temperatures for germination and disease development (22, 32, 33).

It seems that since nocturnal temperatures (Figs. C, 4-9) fall within the range necessary for sporulation (10-25C) of P. manshurica (17, 30), that it is the nighttime RH which plays a major role in the amount of conidia released the following morning. When the RH was 100% for several hours, the numbers of conidia liberated was high (Figs. A, B, 4-6). On the other hand, in the case of 11-14 September (Fig. 7A, B), the RH was not above 90% during this time period and thus, no conidia were observed. In the case of 17-19 September (Fig. 8A, B), the RH was at 100% for more than 48 hours, but no conidia were observed. This is due to the fact that without a decrease in RH, there is no conidial release. If the RH does not drop, the conidiophores will not dry down, thus no release of conidia (23).

These observations suggest that the number of hours in which the RH was approximately 100% during the night is the limiting factor in the degree of sporulation and thus,

numbers of conidia released the following morning. However, when the number of hours at optimum RH versus numbers of conidia released was analyzed statistically, the correlation coefficient was very low (ie, 0.128). This suggests a very poor relationship between humidity and conidial liberation. Cruickshank (5) on the other hand, has shown with studies conducted on P. tabacina that it is not a simple relationship which exists between onset of optimum humidity conditions and time of sporulation. The study on sporulation shows that optimum conditions should occur 3 hr prior to 0500hr for sporulation to occur. Likewise, it shows that more than 3 hr at optimum conditions prior to 0500 hr had very little of an additive effect on the intensity of sporulation. This relationship would explain why a few hours of optimum conditions had the same effect as several hours. Cruickshank (5) also shows that a decrease in the RH below approximately 95% during this critical period would greatly reduce the intensity of sporulation. On the other hand, Cruickshank (5) also shows that a decrease in the RH after 0500 hr did not reduce the sporulation. This was explained by the fact that the sporulation process is in two parts. The first part is conidiophore development and the second part is conidia formation. It appears that conidiophore development is dependent on optimum humidity conditions. Any drop in the humidity before this development is complete will inhibit conidia formation. If, how-

ever, conidiophore development has progressed to a certain stage when the humidity decreases, conidial formation will continue regardless of the humidity. So it would seem that it is not the length of time in which optimum RH occurs, but during what period of time it occurs.

Fungicide Tests. It would be very difficult to state that one fungicide out-performed another for several reasons (Table 3): First, the plots used in these tests many times had poor stands due mainly to disease and insect problems. Second, weeds were a constant problem throughout the growing season, with some areas worse than others. Third, two of the varieties (Alamo and V-1) are not very well adapted to this area. These two varieties are of a Group IX maturity classification. The College Station area is simply too far north for these varieties to grow well.

Currently soybean downy mildew is not a major disease problem in the major soybean growing area of the U.S. However, if this disease should become of major importance, it could be controlled in two basic manners by either resistant varieties or by the use of fungicide. In the area of fungicides, one particular fungicide which was used in this study which may prove to be useful is CGA 48988 (Rid-o-mil). Rid-o-mil has exhibited excellent activity against another downy mildew organism (ie, sorghum downy mildew). Rid-o-mil is a systemic fungicide which has proven effective against

sorghum downy mildew in both seed treatment and foliar sprays. The main advantages of Rid-o-mil are that it is curative in its method of control and requires a small amount per acre (0.14 kg ai/ha). Rid-o-mil may prove itself more effective than the others, but it will require much more testing than what was done in this study.

CONCLUSIONS

Relative humidity is probably one of the major factors contributing to the process of sporulation and conidial release. High humidity is necessary for sporulation of P. manshurica. This optimum humidity must occur during a critical time for abundant sporulation. A decrease in the relative humidity is necessary for conidial release. This decrease causes a drying and twisting of the conidiophore, as in other downy mildews. The twisting of the conidiophore causes the conidia to be ejected and, thus, become air-borne.

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APPENDIX

Table 1A. Spore collection and climatic data collected at the soybean downy mildew nursery during 20 August to 24 August, 1979 (2 hr. intervals).

Day	Hour	Spore	Corrected spore[a]	Humidity (%)	Temperature (C)
20 Aug.	8	1	2 d	80	23.3
	10	23	49a	51	34.4
	12	19	48a	34	35.5
	14	13	40ab	31	36.6
	16	8	34 bc	37	36.1
	18	4	31 c	53	36.1
	20	2	2 d	79	28.8
	22	0	0 d	91	27.2
	24	0	0 d	98	24.4
	2	0	0 d	100	23.3
	4	0	0 d	100	22.7
	6	0	0 d	100	22.2
21 Aug.	9	15	30 d	63	27.2
	11	24	56a	40	32.2
	13	15	42 b	32	35.3
	15	11	40 b	30	36.6
	17	6	34 c	35	36.6
	19	4	4 e	61	35.0
	21	2	1 ef	71	28.3
	23	1	1 ef	80	27.7
	1	0	0 f	90	25.0
	3	0	0 f	100	24.4
	5	0	0 f	100	23.3
	7	0	0 f	73	22.7
22 Aug.	8	1	2 d	94	20.0
	10	27	58a	90	21.1
	12	20	51a	55	26.6
	14	13	40 b	51	30.5
	16	7	29 c	50	32.2
	18	3	23 c	55	31.6
	20	2	2 d	66	27.7
	22	1	1 d	82	25.0
	24	0	0 d	100	18.3
	2	0	0 d	100	19.4
	4	3	5 d	88	20.5
	6	1	2 d	83	20.0
23 Aug.	9	13	26 b	65	23.8
	11	18	42a	47	24.4
	13	0	0 c	43	29.4
	15	0	0 c	41	32.7
	17	0	0 c	47	33.3
	19	3	3 c	70	31.6
	21	2	2 c	90	25.0
	23	0	0 c	100	23.8
	1	2	3 c	100	22.7
	3	2	3 c	100	21.6
	5	1	2 c	100	21.6
	7	2	4 c	100	21.1
24 Aug.	8	12	22 c	100	18.8
	10	56	120 b	61	26.6
	12	105	267a	46	31.1
	14	35	109 b	36	33.3
	16	29	123 b	62	34.4
	18	37	287a	75	26.6
	20	8	8 c	86	25.5
	22	3	3 c	94	22.7
	24	3	4 c	100	21.6
	2	1	1 c	100	20.5
	4	2	3 c	100	20.0
	6	2	3 c	100	19.4

[a] Means in a column followed by the same letter do not differ significantly (P=0.05) by Duncan's multiple range test. Duncan's multiple range test was conducted only on hours within each day.

Table 2A. Spore collection and climatic data collected at the soybean downy mildew nursery during 27 August to 31 August, 1979 (2 hr. intervals).

Day	Hour	Spore	Corrected spore[a]	Humidity (%)	Temperature (C)
27 Aug.	9	21	42 b	70	25.5
	11	28	65a	48	30.5
	13	15	42 b	43	33.3
	15	3	17 c	47	32.7
	17	0	0 c	40	33.8
	19	3	3 c	64	32.2
	21	4	4 c	85	25.0
	23	0	0 c	92	24.4
	1	0	0 c	100	23.3
	3	0	0 c	100	22.2
	5	1	2 c	100	21.6
	7	7	12 c	100	22.7
28 Aug.	8	6	11 de	100	22.2
	10	36	77a	55	28.3
	12	26	66ab	44	32.2
	14	14	44 bc	38	35.0
	16	7	30 cd	36	36.6
	18	6	47 bc	47	33.8
	20	5	5 e	83	27.7
	22	0	0 c	98	24.4
	24	0	0 c	100	22.7
	2	0	0 c	100	22.2
	4	0	0 c	100	22.2
	6	0	0 c	100	22.2
29 Aug.	9	23	46 b	86	25.5
	11	99	231a	60	30.0
	13	1	3 b	94	26.6
	15	4	15 b	71	29.4
	17	1	6 b	95	28.8
	19	1	1 b	97	24.4
	21	1	1 b	100	24.4
	23	0	0 b	100	23.8
	1	1	1 b	100	22.7
	3	1	2 b	100	22.2
	5	1	2 b	100	21.6
	7	0	0 b	100	21.6
30 Aug.	8	1	2 e	90	22.7
	10	540	1161 b	90	22.7
	12	589	1496a	61	27.7
	14	288	896 c	60	32.2
	16	84	356 d	50	33.8
	18	12	93 e	61	33.8
	20	23	24 e	82	31.1
	22	20	22 e	75	27.7
	24	7	8 e	88	26.6
	2	12	16 e	95	25.5
	4	6	9 e	98	24.4
	6	1	2 e	100	22.7
31 Aug.	9	70	140 c	100	23.8
	11	109	254 b	66	28.8
	13	49	137 c	59	31.1
	15	24	88 c	48	33.8
	17	161	902a	49	33.8
	19	2	2 d	62	30.5
	21	4	4 d	84	27.7
	23	2	2 d	88	25.5
	1	0	0 d	97	24.4
	3	0	0 d	98	23.8
	5	0	0 d	100	27.7
	7	0	0 d	100	22.7

[a] Means in a column followed by the same letter do not differ significantly (P=0.05) by Duncan's multiple range test. Duncan's multiple range test was conducted only on hours within each day.

Table 3A. Spore collection and climatic data collected at the soybean downy mildew nursery during 3 September to 7 September, 1979 (2 hr. intervals).

Day	Hour	Spore	Corrected spore	Humidity (%)	Temperature (C)
3 Sept.	8	0	0 d[a]	100	22.7
	10	33	71a	50	27.2
	12	30	76a	47	32.7
	14	15	47 b	62	33.8
	16	9	38 b	82	32.2
	18	2	16 c	76	28.3
	20	3	3 d	89	78.8
	22	1	1 d	100	26.1
	24	0	0 d	100	25.0
	2	0	0 d	98	23.8
	4	0	0 d	99	22.7
	6	0	0 d	100	22.7
4 Sept.	9	32	64 b	100	23.8
	11	35	82a	72	27.7
	13	22	61 b	55	29.4
	15	6	22 c	53	37.2
	17	4	22 c	60	30.5
	19	1	1 d	66	27.7
	21	1	1 d	95	25.0
	23	0	0 d	98	23.8
	1	0	0 d	99	22.7
	3	0	0 d	100	22.2
	5	0	0 d	100	21.6
	7	0	0 d	100	22.2
5 Sept.	8	0	0 c	100	22.7
	10	155	333a	91	25.0
	12	69	175 b	52	31.6
	14	10	31 c	42	34.4
	16	5	21 c	44	34.4
	18	3	23 c	65	31.1
	20	1	1 c	92	26.1
	22	0	0 c	93	23.8
	24	0	0 c	96	23.3
	2	0	0 c	98	22.7
	4	0	0 c	100	22.2
	6	0	0 c	100	22.7
6 Sept.	9	14	28ab	100	23.3
	11	17	40a	67	28.3
	13	8	22 bc	44	33.8
	15	5	18 bc	54	31.6
	17	2	11 cd	51	33.3
	19	0	0 d	74	27.2
	21	0	0 d	88	24.4
	23	0	0 d	95	23.3
	1	0	0 d	98	21.6
	3	0	0 d	100	20.5
	5	0	0 d	100	20.5
	7	0	0 d	100	20.5
7 Sept.	8	0	0 c	93	21.1
	10	16	34a	91	24.4
	12	13	33a	54	30.5
	14	7	22 b	45	33.3
	16	4	17 b	38	34.4
	18	3	23 b	43	33.3
	20	2	2 c	52	29.4
	22	0	0 c	78	25.5
	24	0	0 c	94	22.7
	2	0	0 c	98	22.2
	4	0	0 c	98	21.6
	6	0	0 c	98	21.1

[a]Means in a column followed by the same letter do not differ significantly (P=0.05) by Duncan's multiple range test. Duncan's multiple range test was conducted only on hours within each day.

Table 4A. Spore collection and climatic data collected at the soybean downy mildew nursery during 10 September to 14 September, 1979 (2 hr. intervals).

Day	Hour	Spore	Corrected spore	Humidity (%)	Temperature (C)
10 Sept.	9	4	8 b[a]	100	23.3
	11	3	7 c	61	28.3
	13	4	11a	40	32.7
	15	1	4 d	32	33.3
	17	1	6 c	38	31.6
	19	0	0 e	43	28.8
	21	0	0 e	64	23.8
	23	0	0 e	81	22.7
	1	0	0 e	88	21.6
	3	0	0 e	94	21.6
	5	0	0 e	98	21.6
	7	0	0 e	100	21.6
11 Sept.	8	0	0a	82	22.2
	10	0	0a	80	26.6
	12	0	0a	52	32.7
	14	0	0a	35	32.7
	16	0	0a	30	32.7
	18	0	0a	36	29.4
	20	0	0a	47	24.4
	22	0	0a	68	22.7
	24	0	0a	74	22.7
	2	0	0a	82	22.7
	4	0	0a	82	22.2
	6	0	0a	82	22.2
12 Sept.	9	0	0a	79	23.8
	11	0	0a	51	27.7
	13	0	0a	43	32.7
	15	0	0a	37	32.2
	17	0	0a	46	30.5
	19	0	0a	51	27.7
	21	0	0a	61	24.4
	23	0	0a	68	23.8
	1	0	0a	74	22.7
	3	0	0a	76	22.7
	5	0	0a	80	21.6
	7	0	0a	81	21.6
13 Sept.	8	0	0a	89	20.5
	10	0	0a	72	26.1
	12	0	0a	49	28.8
	14	0	0a	37	32.7
	16	0	0a	33	32.7
	18	0	0a	34	32.2
	20	0	0a	44	26.6
	22	0	0a	59	24.4
	24	0	0a	72	22.7
	2	0	0a	77	21.6
	4	0	0a	85	20.5
	6	0	0a	87	20.5
14 Sept.	9	0	0a	77	22.2
	11	0	0a	50	26.1
	13	0	0a	44	28.8
	15	0	0a	38	30.5
	17	0	0a	33	30.5
	19	0	0a	42	27.7
	21	0	0a	52	22.7
	23	0	0a	59	20.5
	1	0	0a	70	18.8
	3	0	0a	80	16.6
	5	0	0a	81	16.1
	7	0	0a	81	20.0

[a]Means in a column followed by the same letter do not differ significantly (P=0.05) by Duncan's multiple range test. Duncan's multiple range test was conducted only on hours within each day.

Table 5A. Spore collection and climatic data collected at the soybean downy mildew nursery during 17 September to 21 September, 1979 (2 hr. intervals).

Day	Hour	Spore	Corrected spore	Humidity (%)	Temperature (C)
17 Sept.	8	0	[a] 0a	100	21.1
	10	0	0a	90	23.3
	12	0	0a	57	25.5
	14	0	0a	51	28.8
	16	0	0a	68	27.7
	18	0	0a	81	25.5
	20	0	0a	100	23.3
	22	0	0a	100	22.2
	24	0	0a	100	21.1
	2	0	0a	100	21.1
	4	0	0a	100	21.1
	6	0	0a	100	21.1
18 Sept.	9	0	0a	100	20.0
	11	0	0a	100	20.0
	13	0	0a	100	18.8
	15	0	0a	100	18.8
	17	0	0a	100	18.8
	19	0	0a	100	18.8
	21	0	0a	100	18.8
	23	0	0a	100	20.0
	1	0	0a	100	20.0
	3	0	0a	100	21.1
	5	0	0a	100	20.0
	7	0	0a	100	20.0
19 Sept.	9	0	0a	100	20.2
	11	0	0a	100	20.5
	13	0	0a	100	20.5
	15	0	0a	100	19.4
	17	0	0a	100	19.4
	19	0	0a	100	20.0
	21	0	0a	100	19.4
	23	0	0a	100	19.4
	1	0	0a	100	19.4
	3	0	0a	100	19.4
	5	0	0a	100	19.4
	7	0	0a	100	19.4
20 Sept.	8	0	0a	100	16.6
	10	2	5 c	100	20.0
	12	5	13 b	89	21.1
	14	12	37a	80	27.2
	16	3	13 b	69	23.3
	18	0	0 d	71	24.4
	20	0	0 d	69	22.7
	22	0	0 d	89	20.0
	24	0	0 d	100	18.3
	2	0	0 d	100	17.7
	4	0	0 d	100	17.7
	6	0	0 d	100	16.6
21 Sept.	9	20	40a	89	17.2
	11	15	35a	53	21.1
	13	10	28 b	40	25.0
	15	4	15 c	39	26.6
	17	2	11 c	37	27.7
	19	0	0 d	45	27.2
	21	0	0 d	64	23.3
	23	0	0 d	100	19.4
	1	0	0 d	100	17.7
	3	0	0 d	100	17.2
	5	0	0 d	100	16.6
	7	0	0 d	100	16.1

[a]Means in a column followed by the same letter do not differ significantly (P=0.05) by Duncan's multiple range test. Duncan's multiple range test was conducted only on hours within each day.

Table 6A. Spore collection and climatic data collected at the soybean downy mildew nursery during 24 September to 28 September 1979 (2 hr. intervals).

Day	Hour	Spore	Corrected spore	Humidity (%)	Temperature (C)
24 Sept.	8	0	0 d[a]	100	13.3
	10	7	15a	78	21.6
	12	6	16a	56	26.6
	14	3	9 b	45	29.4
	16	1	4 c	43	30.0
	18	2	16a	35	29.1
	20	0	0 d	49	23.3
	22	0	0 d	80	20.0
	24	0	0 d	91	17.7
	2	0	0 d	100	16.6
	4	0	0 d	100	14.4
	6	0	0 d	100	13.3
25 Sept.	8	4	8 b	100	16.1
	10	6	14a	63	22.2
	12	3	8 b	43	27.2
	14	2	8 b	39	28.3
	16	1	6 b	34	29.4
	18	0	0 c	42	27.2
	20	0	0 c	78	20.0
	22	0	0 c	94	17.7
	24	0	0 c	95	16.6
	2	0	0 c	96	16.6
	4	0	0 c	97	13.5
	6	0	0 c	99	15.5
26 Sept.	8	0	0 b	100	16.6
	10	4	9a	87	21.1
	12	3	8a	63	26.6
	14	3	10a	51	29.4
	16	3	8a	45	30.5
	18	1	8a	42	30.5
	20	0	0 b	61	25.0
	22	0	0 b	83	20.5
	24	0	0 b	99	19.4
	2	0	0 b	100	18.3
	4	0	0 b	100	17.2
	6	0	0 b	100	16.6
27 Sept.	8	4	8ab	100	17.2
	10	4	9a	70	23.3
	12	3	9a	58	28.3
	14	2	8ab	45	30.5
	16	1	6 b	42	31.1
	18	1	1 c	45	30.0
	20	1	1 c	67	23.8
	22	0	0 c	98	20.0
	24	0	0 c	85	20.0
	2	0	0 c	92	18.8
	4	0	0 c	98	17.7
	6	0	0 c	100	17.7
28 Sept.	8	0	0 d	100	20.5
	10	5	11a	81	20.3
	12	4	10a	57	25.5
	14	2	5 c	45	29.4
	16	2	3 c	43	30.3
	18	1	3 c	43	31.1
	20	0	0 d	71	28.3
	22	0	0 d	87	23.8
	24	0	0 d	95	22.2
	2	0	0 d	100	20.3
	4	0	0 d	100	20.0
	6	0	0 d	100	20.0

[a] Means in a column followed by the same letter do not differ significantly (P=0.05) by Duncan's multiple range test. Duncan's multiple range test was conducted only on hours within each day.

VITA

David Brian Madden, son of Mr. and Mrs. Frank H. Madden, was born August 20, 1956, in Houston, Texas. He graduated from J. Frank Dobie High School in 1974. In 1974, he entered Texas A&M University with a major in Biology. In 1978, he received a Bachelor of Science degree with a major in Botany. In 1978, he enrolled in the graduate program at Texas A&M University and at the time of this writing, he was a candidate for a Master of Science degree in Plant Pathology.

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