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Downy mildew of soybeans

Vernyl Duwaine Pederson

Iowa State University

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DOWNY MILDEW OF SOYBEANS

by

Vernyl Duwaine Pederson

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
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In Charge of Major Work

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Head of Major Department

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Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

1961
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INTRODUCTION

Downy mildew of soybeans caused by *Peronospora manshurica* (Naom.) Syd. ex Gaum. occurs throughout the soybean-growing areas of the world. Despite the widespread occurrence and destructive potential of downy mildew, detailed investigation has not been made on many aspects of the etiology or epiphytology of the disease. Major research efforts have been directed toward determination of races and varietal reactions to isolates of the fungus.

The disease may appear early during the soybean-growing season, building up rapidly by air-borne secondary inoculum. Therefore, large acreages of susceptible varieties encourage the increase of virulent races of the pathogen. With increases in soybean acreage in recent years, the study of downy mildew has become increasingly important. Control at present depends upon an active breeding program to develop varieties resistant to prevalent and damaging races of the pathogen. Effective progress in obtaining control requires knowledge of the factors influencing development of the disease and acquisition of techniques for obtaining and maintaining isolates of the fungus.

The objectives of this research, involving three separate but interrelated aspects of the development of downy mildew, are as follows:

1. To investigate factors which influence systemic in-
2. To investigate physical and biological aspects of conidial germination.

3. To investigate the effect of environment on host-pathogen interaction.
Peronospora manshurica, the fungus causing downy mildew of soybeans, was first described on soybeans in Manchuria in 1921 (Murai 1921). A year later, it was reported on soybeans in the United States (Haskill and Wood 1923) and subsequently identified in all of the soybean growing areas of United States and several parts of the world (Hildebrand and Koch 1951).

Despite the widespread prevalence and consistent occurrence of the disease on commercially grown susceptible varieties of soybeans, little work has been done on the life history of the organism. Some of the pertinent phases have been investigated, however, and these will be discussed in sequence with aspects related to the investigation of other downy mildews which are applicable to the experiments herein reported.

Primary infection is initiated by oospores which may be seed-borne as a milky-white encrustation on the surface of the seed (Wolf and Lehman 1924, 1926; Johnson and Lefebvre 1942), or soil-borne with infected leaf and pod debris (Hildebrand and Koch 1951). Systemic infection of soybean seedlings may occur when oospore-encrusted seeds are planted (Hildebrand and Koch 1945, Koch and Hildebrand 1946, Jones and Torrie 1946). The fungus sporulates profusely from the lower surfaces of leaves of such systemically infected plants.
(Hildebrand and Koch 1945, 1951), thus providing a source of initial inoculum for spread of the organism in the field.

Yield of systemically infected plants for greenhouse or laboratory studies may be increased by removing oospores from an encrusted seed, diluting them with talc, or suspending them in water and placing them between cotyledons of germinating seedlings of susceptible varieties of soybeans (Grabe and Dunleavy 1959).

Various factors affecting systemic infection have been studied (Grabe 1957). There is some indication that pre-chilling moistened, encrusted seeds for 6 days at 10 C before planting results in more systemic infection than planting encrusted seed directly in soil.

All attempts to germinate oospores of P. manshurica have failed (Grabe 1957). Investigators have experienced difficulty in obtaining germination of oospores of other members of the Peronosporaceae. Those that have observed germination have not been able to define factors which influence it. Adequate aeration and exposure to water for periods of time appear to be associated with the germination of oospores (Gregory 1912, Hiura 1930, Howe 1930, Evans and Harrar 1930, Chaudhuri 1932, Tasugi 1933, McDonough 1936, Wolf et al. 1936). The significance of these factors has not been established, however.

After a primary outbreak of downy mildew in a field of
soybeans, secondary spread of the disease depends on a number of environmental conditions. These conditions, such as light, temperature, humidity, rainfall, dew, and wind, are all involved in sporulation, spore discharge, dispersal, germination, and reinfection. A thorough study of climatic factors in relation to secondary spread of *P. manshurica* has not been made, but certain investigations concerning these factors and other downy mildews are applicable.

**Sporulation**

Abundant moisture with long periods of high relative humidity and cool night temperatures are the general pre-requisites for abundant sporulation of the downy mildews (Cook 1932, Dixon et al. 1936, Clayton and Gaines 1945). Differentiation in the type of deposition of moisture, whether by dew or rain, is not often made. Weston (1923), however, observed that heavy dews were more conducive to sporulation of *Sclerospora* sp. in maize than mist or rain. Sporulation generally occurs during the early morning hours and the spores become mature by sunrise (McGrath and Miller 1958).

Yarwood (1937) studied the diurnal cycle of sporulation of several downy mildews and determined that light, as well as high moisture, was an important factor. The fungus did not sporulate when infected leaves were placed in a moist chamber after having been in the dark for 12 hours. He con-
cluded that a more basic cause of sporulation was the metabolic state of the host and parasite conditioned by a sufficient period of light immediately before the plants were placed in a moist chamber.

**Spore Discharge and Dispersal**

The significance of early morning spore discharge in the downy mildew fungi was anticipated by de Bary (1887) when he described the spore discharge mechanism of *Phytophthora infestans*:

Where filiform sporophores rise free into the air, a further mechanical arrangement is found which greatly assists the shedding and scattering of the abscised spores. It may be readily observed in the Hyphomycetes, in Peronospora, for example, *Phytophthora infestans*, and in the gonidiophores of *Peziza Fucelliana*, etc. The hyphae of these fungi are cylindrical in the moist and turgescent state, but collapse when dry and especially when the spores are ripe into a flat ribbon-like form, and the drier they are the more strongly do they become twisted round their own longitudinal axis. They are so highly hygroscopic that the slightest change in the humidity of the surrounding air, such for instance as may be caused by the breath of the observer, at once produces changes in their turgescence and torsion; the latter give a twirling motion to the extremity of the gonidiophore and the ripe spores are thereby thrown off in every direction.

Spore trapping procedures have verified the conclusion that downy mildew conidia are disseminated following, not during, a period of continuous moisture saturation (Pinckard 1942, Hirst 1953, Waggoner and Taylor 1957, 1958). Cruickshank (1958) found that maximum spore discharge occurred when
the humidity dropped from 100 to 40%.

After discharge from the conidiophores, conidia of downy mildew are disseminated widely by wind currents. Viable conidia of *P. destructor* have been trapped at least 1500 feet above diseased onion beds (Newhall 1938). Viable conidia of *P. tabacina* have been disseminated by the wind 20 miles from a source of inoculum in tobacco beds (Hyre 1952).

Conidial Germination

Conidia, during the period of dissemination, are subject to various climatic forces which determine whether a high proportion of them will germinate and cause secondary infection. The majority of workers indicate conidia must fall on susceptible leaves, germinate, and cause infection within a few hours after being discharged if secondary spread is to be successful (Weston 1923, Cook 1932, Clayton and Gaines 1945, McGrath and Miller 1958). Despite this opinion, other workers have found that conidia remain viable for several days after they are removed from conidiophores (Angell and Hill 1931, Wolf *et al.* 1934, Newhall 1938). Yarwood (1943) simulated natural conditions as closely as possible in determining longevity of conidia of the onion downy mildew fungus by allowing them to remain intact on the leaves of plants. He concluded that sporangia may live three to five days on sporangiophores of turgid leaves intact on plants,
but may live for a shorter time on detached, wilted leaves.

Viability of conidia has been prolonged by storage at low or freezing temperatures (Leach 1930, Angell and Hill 1931) but lyophilization techniques for storage have not been successful (Grabe 1957).

The influence of temperature on conidial germination has been used by some workers to develop hypotheses regarding minimum, maximum, and optimum conditions necessary for development of downy mildew in the field. The effect of temperature on germination has not always been consistent, however (Gregory 1912, Wolf et al. 1934, Newhall 1938, Clayton 1945). Angell and Hill (1932) stated:

Variation in percentage germination (of *Peronospora tabacina*) was noticed again and again during the past three years, and unavailing efforts were repeatedly made to find out the factors that possibly influence it.... No correlation could be obtained between factors of temperature and humidity and viability of conidia.

Various factors in addition to temperature may cause variation in conidial germination. Some examples are: light and reaction of medium (Wolf et al. 1934), time of collection of conidia after sporulation (Clayton 1945), and inclusion of colloidal substances with conidial suspensions (Crosier 1934).

No attempt has been made to correlate germination and concentration of conidia. The association between high concentration and low germination of spores of some of the
saproxylic fungi has been noted (Duggar 1961, Doran 1952); but the cause has not been attributed to the presence of high concentrations of spores per se. Recently, however, low germination of uredospores of some of the rust fungi has been attributed to the presence of the spores themselves. This phenomenon has been described by the general term self-inhibition.

Allen (1955) found that heavy concentrations of uredospores floated on water did not germinate, but would germinate after being transferred to fresh water. Very low concentrations of spores would also germinate on water. The inhibitor could be detected in the water on which heavy masses of spores had floated. From these observations, he concluded that germination inhibition was caused by a water soluble metabolite produced by the spores. Evidence was obtained that the inhibitor was a volatile acid. Forsythe (1955) believed the volatile inhibitor released by the spores was trimethylethylene.

The role of an inhibitor produced by spores themselves in preventing germination of related spores has been investigated by Yarwood (1954a, 1954b, 1956a, 1956b). He proposed that gaseous substances liberated by bean rust uredospores prevented germination of uredospores of a second inoculation of a rust species. Aspartic and glutamic acids were present in sufficient quantities around a rust pustule in bean
leaf tissue to account for protection against reinfection near the pustule (Wilson 1958).
MATERIALS AND METHODS

Race 2 of *Peronospora manshurica*, described by Grabe and Dunleavy (1959), was used for all of the experiments conducted in the laboratory and greenhouse. This race was obtained by collecting conidia from leaves of Hawkeye soybeans grown in the field and transferring them to Illini soybean seedlings grown in the greenhouse. The isolate was maintained on Illini soybean seedlings by transfer every eight to ten days.

Illini soybeans, highly susceptible to Race 2 of *P. manshurica*, were used for most of the experiments conducted in the laboratory and greenhouse. Blackhawk soybeans, also susceptible to Race 2, were grown in field plots and inoculated in the field to provide a source of conidia for field experiments.

Soybean seedlings for isolate maintenance and experimental purposes were grown in autoclaved soil in 4-inch clay pots. Routine inoculation of soybean seedlings was accomplished by spraying plants 10-12 days old with a water suspension of conidia. The plants were placed in a moist chamber for approximately 12 hours at 15-20 C while the leaves were still wet. Thirty gallon metal garbage cans with tight fitting covers, or polyethylene plastic bags large enough to enclose completely a single pot of seedlings, provided con-
venient moist chambers. After removal from the moist chamber, the pots of plants were returned to the greenhouse for the incubation period. During the winter months when most of the experiments were performed, the temperature of the greenhouse varied from a minimum of 18 °C at night to a maximum of 25 °C during the day.

Systemic infection was obtained by inoculating seed of Illini or Hawkeye soybeans with oospores of P. manshurica. The oospores were obtained either from oospore encrusted seed or from dried, infected leaves collected from the field. When oospores from seed were used, they were diluted approximately 1:10 with talc or other diluent and deposited, by means of a small spatula, between the cotyledons of soybean seed from which the seed coat had been removed. Removal of the seed coats was facilitated by soaking the seed for 12-24 hours in water at 8 °C. For each test, 50-100 seeds were inoculated and planted in steamed soil in 4-inch clay pots. The seedlings were grown in a greenhouse in which the temperature ranged from 18-24 °C.

Oospores from leaf debris were used to obtain systemic infection by pulverizing the leaf debris in a mortar. This powder was deposited between the cotyledons of the seed in a manner similar to the method used to obtain systemic infection with oospores from encrusted seed. Other methods of inoculation with leaf debris included planting seeds directly
in a quarter-inch layer of pulverized, infected leaf debris, or subjecting a wet mixture of seed and leaf debris to a vacuum for 30 minutes in a large vacuum desiccator.

Conidia used for investigations on germination were obtained from infected leaves of Illini soybean plants. A conidial suspension was prepared by washing the conidia off leaves into a small quantity of water. The concentration of conidia in suspension was adjusted to about 400 conidia per mm$^3$. This number of conidia per unit volume of water was determined by making percentage light transmission readings through the conidial suspension with a Lumitron colorimeter. The colorimeter was calibrated by making percentage light transmission readings of a series of suspensions of conidia of known concentration. The concentrations of the calibrated suspensions were determined by averaging ten consecutive counts of conidia in each suspension in a haemacytometer. By plotting the number of conidia per cubic millimeter against percentage light transmission, the curve shown in Figure 1 was obtained. A series of six concentrations of conidia was made by diluting the initial conidial suspension in geometric progression. One drop of the conidial suspension from each of the concentrations was placed on the surface of individual water agar discs (Figure 2) and incubated 12 hours for the germination tests. Each concentration was replicated four times. The percentage germination on each disc was deter-
Figure 1. Percentage light transmission through various concentrations of conidia of *P. manshurica* in water
Figure 2.  

A. Arrangement of water agar discs in a petri dish preparatory to incubation of conidia of *P. manshurica* on their surfaces

B. Conidial suspensions of *P. manshurica* on the surfaces of single and double water agar discs
mined from four separate counts of 100 spores each. A uniform volume of agar in each disc was obtained by pouring 25 ml of 2% water agar into flat-bottomed petri dishes and cutting the agar with a cork borer 7 mm in diameter.

Seeds of the susceptible variety Illini and of the resistant variety Woods Yellow were planted in 4-inch pots of steamed soil and grown in a greenhouse for experiments on the effect of light duration on host-parasite interaction. After emergence, the plants were exposed daily to 6, 12, or 18 hours of light. Identical light duration treatments were given to two sets of four replications of each variety. Light duration was regulated by placing corrugated cardboard boxes over the sets of pots. The boxes were insulated by covering them with aluminum foil. The temperature within the boxes did not vary more than 2 C from the temperature at bench level outside the boxes. Duration of light was regulated on a daily schedule as follows: Plants which received six hours of light daily were covered at 3:30 PM and those which received 12 hours of light daily were covered at 9:30 PM. All plants were uncovered at 9:30 AM each day. Incandescent light was used to supplement daylight and provide light for part of the 12- and 18-hour light treatments. The lights were automatically turned on at 5:00 PM daily and off at 1:00 AM the following morning. The maximum brightness on cloudless days averaged 8,000 footcandles from 8:30 AM
to 2:30 PM. Incandescent illumination averaged 200 foot-candles.

The plants were uniformly inoculated with a suspension of conidia ten days after planting. Plants were then returned to their former position on the greenhouse bench and the daily light treatment schedule was resumed. The treatments were continued for 26 days. All of the infected unifoliate leaves were necrotic when the experiment was terminated.

Sporulation determinations were initiated on the evening of the sixth day and, subsequently, every two days thereafter. Leaves were selected at random from the treatments and placed in petri dish moist chambers for 12 hours at 20 C. Amount of sporulation was rated according to an arbitrary scale ranging from 1-5, with 1 indicating no sporulation, 2-very sparse, 3-light, 4-moderate, and 5-heavy sporulation. These observations were continued until no sporulation could be obtained on the leaves selected from a particular light duration treatment.

Determinations of the effect of light duration on areas of infection and intensity of sporulation were made eight days after inoculation. A set of four replications of infected Illini and Woods Yellow seedlings was placed in a moist chamber to obtain sporulation. All unifoliate leaves were removed from the plants in each pot and the conidia were
washed off in 10 ml of water. The density of the spore suspensions was measured photometrically. These values were converted to concentration of conidia per unit area of infected leaf surface. The outline of each leaf and the boundaries of visible symptoms were recorded by making three diameter enlarged tracings with a pantograph. The respective outlined areas were determined with a planimeter.

Studies of sporulation, conidial dispersal, longevity, and infection associated with field conditions were conducted over a period of 17 days in August, 1959. Methods of study were chosen that would emphasize the application of principles discovered in greenhouse and laboratory studies.

Hawkeye seedlings were placed in a field of Blackhawk soybeans where downy mildew had been established by artificial inoculation. Eight pots containing four uniform plants per pot were placed in the field for the same length of time. Data consisted of the total number of infection loci that developed on unifoliate leaves ten days after the seedlings were exposed to the field conditions.

Three procedures were used for placement of seedlings in the field. For the first procedure, all pots of seedlings were placed in the field in the evening and removed, eight pots at a time, at intervals throughout the following day. Four of the pots removed at each interval were covered with plastic bag moist chambers in order to insure germination and
infection by the conidia that may have fallen on the leaves during the exposure period in the field. The other four were left uncovered to determine whether the conditions of moisture or humidity for the field exposure period were sufficient to permit germination and infection. After 24 hours at 20 C, the plants were placed in a greenhouse. This procedure was designed to determine the period of time during which conidial discharge was most intense, the longevity of conidia on test plants remaining in the field for various intervals of time, and the probability of infection by conidia during the test period.

For the second procedure, test plants were placed in the field at intervals throughout the day and removed the following morning at 6:00 AM. This procedure was used primarily to determine whether the night conditions in the field were suitable for germination of conidia which may have fallen on the leaves during the preceding day. This procedure also provided a check on longevity of conidia that fell on plants during the day.

For the third procedure, plants were placed in the field for approximately two-hour periods, removed, and covered immediately with plastic bags. This procedure was designed to determine the number of conidia which fell per hour on the test seedlings in the field.

Temperature, humidity, and dew deposition were recorded
each day of the experimental period. General observations on quantity and persistence of dew, wind velocity, rainfall, and cloud cover were made each time plants were placed in or taken out of the field.

Because of the diverse nature of the experimental methods used for the various investigations, specialized methods are described with the results of each experiment.
EXPERIMENTAL RESULTS

Systemic Infection

**Signs and symptoms of systemic infection**

Oospore-encrusted seeds planted in steamed soil and germinated at 18-21 C in the greenhouse usually produced a few systemically infected plants. The first symptoms of systemic infection usually were clearly defined, lighter green areas on the lamina near the petiole. The infection quickly spread along the midvein and into the lamina until most of the leaf surface was involved (Figure 3). Sporulation could be induced seven to ten days after the first visible evidence of infection by placing the plants in a moist chamber for 12 hours.

Visible leaf symptoms did not develop on all infected seedlings. However, infection became evident on some 12-14 day-old plants when sporulation occurred on the surfaces of one or both of the cotyledons after the plants were placed in a moist chamber.

Signs and symptoms of systemic infection usually were evident on one or the other of the cotyledons or unifoliolate leaves, but occasionally both cotyledons and both unifoliolate leaves were involved. At times, the cotyledons were infected but symptoms did not appear in any other parts of the plant. Conversely, symptoms of the disease sometimes appeared on the
Figure 3. Early (right) and advanced (left) stages in the development of symptoms of systemic infection by *Peronospora manshurica* on unifoliate leaves of Illini soybean seedlings.
unifoliate leaves without the cotyledons showing signs of infection.

Symptoms of the disease appeared on the trifoliate leaves as they developed. Many of these leaves developed symptoms similar to those described for the unifoliate leaves; however, on some, symptoms appeared as a mottled, gray-green color. All of the infected trifoliate leaves were conspicuous by their dwarfed appearance.

In contrast to local infection in which the damage to a soybean plant as a whole was slight, systemic infection caused a marked stunting of all aerial organs. Stems were spindly, internodes were shorter, and leaves were smaller and narrower than normal (Figure 4).

Inoculation of soybean seeds with oospores

Oospores scraped from encrusted seed and oospores in infected leaf debris provided sources of inoculum for obtaining systemic infection of soybean seedlings. Low and irregular percentages of infected seedlings often were obtained by inoculating seed with oospores. Therefore, a series of experiments was performed to investigate factors which affect oospore germination and subsequent systemic infection.

Experiment 1. The effect of storage conditions on oospore inoculum and placement of inoculum relative to seeds

Systemic infection occurred when leaf material used as in-
Figure 4. Illini soybean plants of equal age systemically infected (left) and locally infected (right) by *P. manshurica*.
oculum was stored up to five months either indoors or outdoors (Table 1). Exposure of oospores to freezing and thawing was not required for their germination. Fourteen percent of the seedlings were infected when the leaf material stored indoors or outdoors was used for inoculum. No infection resulted when frozen leaf material was used as inoculum.

One plant in 39 was infected when the inoculum was placed on the outside of the seed with the seed coat removed; whereas 14 percent infected seedlings resulted when inoculum was deposited between the cotyledons. No infection resulted when the leaf material was placed on seeds which did not have the seed coats removed. Results of this experiment emphasize the importance of location of inoculum in relation to the seed.

Experiment 2. Depth of planting and inoculum placement relative to seeds

Infected leaf debris collected from between rows of field-grown Blackhawk soybean plants was dried, pulverized, and stored one month at room temperature before it was used for inoculum. Illini seeds were planted in a quarter-inch layer of the debris, at depths of 1 and 3 inches in steamed soil. Eight percent of the plants from seed planted 1 inch deep were systemically infected if the seed coats were removed. Three percent of the intact seed produced systemically infected plants. However, when seeds were planted 3 inches deep, 3% systemic infection resulted
Table 1. Percentage infected Blackhawk soybean seedlings resulting from inoculating seed with oospores of *E. manshurica* in leaf debris stored at -10°C, indoors and outdoors. Experiment 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>-10°C</th>
<th>Oospores stored</th>
<th>Indoors</th>
<th>Outdoors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of seedlings infected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed coat intact</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed coat removed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Leaf debris containing oospores deposited between the cotyledons | 40 | 0 | 43 | 14 | 37 | 14 |
| Moistened seed covered with leaf debris containing oospores   |     |   |    |    |    |    |
| Seed coat intact                                              | -  | - | -  | -  | 44 | 0  |
| Seed coat removed                                             | -  | - | -  | -  | 39 | 2  |

*Number of seedlings from five lots of ten seeds each.*
when the seed coats were removed prior to planting. None became infected when the seed coats were left intact (Table 2).

Results of the preceding experiments indicated contact with the cotyledons or embryo, unprotected by the seed coat,

Table 2. Percentage systemically infected Illini soybean seedlings resulting from seed planted in a quarter-inch layer of leaf debris containing oospores of P. manshurica. Experiment 2

<table>
<thead>
<tr>
<th>Planting depth</th>
<th>Seed coat intact</th>
<th>Seed coat removed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of seedlings</td>
<td>Percent infected</td>
</tr>
<tr>
<td>1 inch</td>
<td>33</td>
<td>3</td>
</tr>
<tr>
<td>3 inches</td>
<td>24</td>
<td>0</td>
</tr>
</tbody>
</table>

was important for successful infection by germinating oospores. Therefore, in the following experiment, a vacuum method was employed as an inoculation technique.

Experiment 3. The use of the vacuum method for inoculation of seeds. Finely pulverized leaf debris containing numerous oospores was suspended in water to which 0.5% Tween-20 (polyoxyethylene sorbitan monolaurate) was added. Illini soybean seeds were prepared for inoculation by soaking them in water for 24 hours. The seed coats were removed from one lot of the seed and left intact on another. The seeds
were mixed with the leaf debris in large vacuum desiccators, then subjected to a vacuum for 20 minutes. Seed inoculated by the vacuum method produced 12% systemically infected seedlings when the seed coats were removed and 9% when the seed coats were left intact (Table 3).

Table 3. Percentage systemically infected Illini soybean seedlings resulting from the use of the vacuum method of inoculation with pulverized, infected soybean leaf debris. Experiment 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of seedlings</th>
<th>Percent infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed coat intact</td>
<td>47</td>
<td>9</td>
</tr>
<tr>
<td>Seed coat removed</td>
<td>42</td>
<td>12</td>
</tr>
</tbody>
</table>

*Number of seedlings from five lots of ten seeds each.

Experiment 4. Effect of varying concentrations of carbon dioxide or oxygen in the atmospheres in which inoculated seeds germinated. Illini soybean seeds were inoculated with an oospore-talc mixture and placed in large vacuum desiccators in which the percentages of carbon dioxide and oxygen were varied. Carbon dioxide at concentrations of 2, 25, and 100% was used for one series of treatments. For another series, oxygen and nitrogen were mixed to give 5, 20, 50, and 100% oxygen. The seeds were allowed to germinate on moist blotters in desiccators for 72 hours at room tempera-
ture prior to planting in soil in the greenhouse. No systemically infected seedlings grew from seeds germinated in various concentrations of carbon dioxide. From 3-10% grew from seeds germinated in various concentrations of oxygen (Table 4). Varying oxygen concentration did not increase systemic infection; higher than normal carbon dioxide completely eliminated it.

Table 4. Percentage Illini soybean seedlings systemically infected by P. manshurica when seeds inoculated with oospores mixed with talc were germinated 72 hours at 20 C in atmospheres of various percentages of oxygen. Experiment 4

<table>
<thead>
<tr>
<th>Percentage oxygen</th>
<th>Number of seedlings</th>
<th>Percent infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>40</td>
<td>3</td>
</tr>
<tr>
<td>20</td>
<td>50</td>
<td>10</td>
</tr>
<tr>
<td>50</td>
<td>40</td>
<td>3</td>
</tr>
<tr>
<td>100</td>
<td>44</td>
<td>9</td>
</tr>
</tbody>
</table>

*Number of seedlings from six lots of ten seeds each.

Experiment 5. Germination of inoculated seeds at four temperatures. Illini seeds were inoculated with an oospore-talc mixture and planted either in autoclaved soil or sand and left for five days in temperature-regulated chambers at 15, 20, 25, and 30 C. After germination, they were returned to the greenhouse for incubation. Temperatures
of 15 and 20°C were more favorable for systemic infection than the higher temperatures. In steamed soil, 30% of the seedlings were infected at the lower temperatures; whereas, 7% and 12% were obtained at 25 and 30°C, respectively. Systemic infection, which developed when inoculated seeds were planted in sand and in soil, was approximately the same (Table 5).

Experiment 6. Inoculation of seeds with oospores mixed with various diluents

A preliminary experiment indicated wide differences in systemic infection resulted from mixing oospores with talc or with non-steamed soil. In this experiment, 21% infection occurred when talc was used to dilute oospores, but no infection occurred when non-steamed soil was used. Another similar preliminary experiment indicated activated, powdered charcoal was somewhat more effective than talc as a diluent. Consequently, these materials, along with 600-mesh carborundum and steamed soil, were mixed with oospores for inoculating soybean seeds. Inoculated seeds were planted in steamed soil and in steamed sand at a depth of 1 inch. Charcoal was most effective and non-steamed soil the least effective as diluents (Table 6). There was no difference in systemic infection when inoculated seeds were planted in steamed soil or in sand. No differences were noted in systemic infection when oospores were mixed with talc, carborundum, or steamed soil.
Table 5. Percentage of systemically infected Illini soybean seedlings from seeds inoculated with oospores mixed with talc and germinated five days at four temperatures in steamed soil and sand. Experiment 5

<table>
<thead>
<tr>
<th>Seed planted in:</th>
<th>Temperature</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15°C</td>
<td>20°C</td>
<td>25°C</td>
<td>30°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of seedlings</td>
<td>Percent infected</td>
<td>Number of seedlings</td>
<td>Percent infected</td>
<td>Number of seedlings</td>
<td>Percent infected</td>
<td>Number of seedlings</td>
</tr>
<tr>
<td>Steamed sand</td>
<td>19</td>
<td>21</td>
<td>18</td>
<td>33</td>
<td>32</td>
<td>3</td>
</tr>
<tr>
<td>Steamed soil</td>
<td>21</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>32</td>
<td>7</td>
</tr>
</tbody>
</table>

\(^a\)Number of seedlings from five lots of ten seeds each.
Table 6. Percentage systemically infected Illini soybean seedlings from seeds inoculated with oospores of *F. manshurica* mixed with various diluents and planted in steamed sand and soil. Experiment 6

<table>
<thead>
<tr>
<th>Seeds planted in:</th>
<th>Activated charcoal</th>
<th>Talc</th>
<th>Carborundum</th>
<th>Steamed soil</th>
<th>Non-steamed soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of infected seedlings</td>
<td>Number of infected seedlings</td>
<td>Number of infected seedlings</td>
<td>Number of infected seedlings</td>
<td>Number of infected seedlings</td>
<td>Number of infected seedlings</td>
</tr>
<tr>
<td>Steamed sand</td>
<td>26</td>
<td>50</td>
<td>24</td>
<td>33</td>
<td>26</td>
</tr>
<tr>
<td>Steamed soil</td>
<td>37</td>
<td>51</td>
<td>33</td>
<td>30</td>
<td>42</td>
</tr>
</tbody>
</table>

*Number of seedlings from five lots of ten seeds each.*
Conidial Germination

Conidia of the fungus *Peronospora manshurica* germinated erratically on the surface of water agar. Factors affecting germination are of interest because of the possible relationship these factors may have to the development of downy mildew of soybeans. The age of the lesion on which conidia are produced, the period of time conidia remain on a leaf before removal, and the period and temperature of storage of the conidia all contribute to germination irregularities. The present investigation arose from attempts to determine the nature of these factors and relate germination potential of conidia under artificial conditions on water agar to infection of soybean seedlings.

The concentration of conidia on the surface of water agar appeared to be the most important factor in controlling germination. Spores in high concentrations germinated poorly, if at all; whereas, germination was improved markedly if the concentration of conidia was decreased (Figure 5). Furthermore, percentage germination was higher when the conidia were located near the edge of a drop of a suspension on water agar in a petri dish than if they were near the center of the drop.

Low germination associated with high concentration of conidia indicated that an inhibitor, diffusible in water agar, was associated with the conidia. This hypothesis was tested in the following experiment.
Figure 5. Germination of four concentrations of conidia of *P. manshurica* at 10 °C on the surface of water agar discs

A. 50 conidia per mm^3; germination 84%
B. 100 conidia per mm^3; germination 65%
C. 200 conidia per mm^3; germination 31%
D. 400 conidia per mm^3; germination 9%
Experiment 7. Effects of concentration of conidia on germination

Conidia in high, medium, and low concentrations were placed on the surface of water agar discs. The discs were turned upside down in a petri dish and another water agar disc was placed over the top of each. A drop of a conidial suspension of the lowest concentration (approximately 12 conidia per mm$^3$) was placed on top of the double disc. Percentage germination of the conidia on the bottom of the two discs and of conidia on the top of the discs was determined after 12 hours incubation at 10 C (Figure 6).

Germination of conidia on the bottom of the lower disc was 7%, 31%, and 58% for the high, medium, and low concentrations, respectively. This compared with germination of 4%, 29%, and 57% on the top of the double disc. The control, consisting of the dilute conidial suspension not in association with conidia on the bottom of the disc, germinated 85%. The inhibitor associated with the conidia on the bottom of the discs apparently diffused through the water agar and controlled germination of conidia on the top of the discs, regardless of their low concentration.

Experiment 8. Germination of conidia on double and single water agar discs

Diffusion of self-inhibitor in water agar from conidia was further tested by comparing germination of various concentrations of conidia placed on the surface of single or double agar discs. Germination was
Figure 6. Percentage germination of low concentrations of conidia of *P. manshurica* incubated 12 hours at 10 C on the top of double water agar discs. Conidia of high (H), medium (M), and low (L) concentrations incubated simultaneously on the bottom of the same double agar discs. Experiment 7
LOCATION OF CONIDIA

TOP OF AGAR DISK

BOTTOM OF AGAR DISK

PERCENTAGE GERMINATION

CONCENTRATION OF CONIDIA
12% on a single disc and 37% on a double disc at the highest concentration of conidia (Figure 7). This indicated that the volume of water agar available for dilution of the inhibitor was also an important factor in determining germination, particularly at the higher concentrations of conidia. The increase in volume of water agar was, therefore, similar in its effect on germination to the decrease in concentration of conidia per unit volume of water.

**Experiment 9. Influence of temperature on germination of conidia**

Conidia were incubated on the surface of water agar discs at 10°C and 20°C to test the hypothesis that the inhibitor was a product of the metabolism of conidia and that its concentration should be influenced by temperature. Percentage germination of conidia at the highest concentration was 12% at 10°C and 33% at 20°C (Figure 8). The effects of higher temperature and dilution of inhibitor were similar, in that germination was increased by both factors. Thus, it appeared that increased germination at 20°C was due to reduced concentration of the inhibitor, possibly by volatilization or inactivation during the incubation period.

**Experiment 10. Germination of conidia after storage in open and closed containers**

Depletion of inhibitor by volatilization or inactivation upon exposure to air was tested by storing 15 ml quantities of conidial suspensions of the
Figure 7. Percentage germination of the conidia of *P. manshurica* at various concentrations after incubation 12 hours at 10 C on (1) double water agar discs, (2) single water agar discs.

Experiment 8
Figure 8. Percentage germination of the conidia of *P. manshurica* at various concentrations after incubation 12 hours on the surface of water agar discs (1) at 20 C, (2) at 10 C. Experiment 9
highest concentration in open beakers or in corked test tubes at 5 C. Germination was determined after 72 hours and after 12 days.

Percentage germination of conidia stored in open beakers for 72 hours was higher than before storage at the higher concentrations (Figure 9). After 12 days storage in open beakers, germination was about 10% at all concentrations (Figure 10). This indicated the effectiveness of the self-inhibitor had been lost. The inhibitor apparently accumulated in corked test tubes, however, because a very low percentage of the conidia that had been stored 72 hours and none that had been stored 12 days germinated. Although no conidia were observed to germinate after storage in closed test tubes for 12 days, infection was readily obtained when they were used as inoculum.

The conidia that were stored in closed test tubes for a length of time exhibited a distinctive, coarse, granular appearance. Conidia not stored but germinated soon after collection from leaves appeared hyaline with a finely granular protoplasm. Conidia with coarse, granular protoplasm did not germinate normally. The contents of some of these conidia appeared to burst through the conidial wall without the formation of a normal germ tube. At times, protoplasm could be seen rapidly changing shape with a churning motion reminiscent of the first stages of zoospore formation in sporangia
Figure 9. Percentage germination of the conidia of *P. manshurica* at various concentrations after incubation 12 hours on the surface of water agar discs at 10°C (1) before storage, (2) after storage in open beakers for 72 hours at 5°C, (3) after storage in corked test tubes for 72 hours at 5°C. Experiment 10
CONIDIA PER CUBIC MILLIMETER

PERCENTAGE GERMINATION

1

2

3
Figure 10. Percentage germination of the conidia of *P. manshurica* at various concentrations after incubation 12 hours on the surface of water agar discs at 10°C (1) before storage, (2) after storage in open beakers for 12 days at 5°C. Experiment 10
of Phytophthora infestans. The contents did not appear to change position or shape after escape from the boundaries of the conidial wall, however (Figure 11).

Conidia having a coarse, granular appearance were much more prevalent when stored in water for various lengths of time in concentrated suspensions. Abnormal conidia appeared sooner and in larger numbers among those stored in water at 20 °C than among those stored at 5 °C (Figure 12). They were also more numerous when stored in corked test tubes than in open beakers. There is no explanation for the occurrence of these abnormal conidia; however, it is possible that inhibitor concentration was a contributing factor.

**Experiment 11. Germination of conidia associated with spore-free filtrates**

Comparison of germination of conidia stored in beakers or in closed test tubes suggested that the inhibitor had accumulated in the water in the test tubes, but had disappeared from the water in the beakers. This hypothesis was tested by allowing water agar discs to imbibe one drop of spore-free filtrate from each of the stored suspensions for 12 hours, then observing germination of a fresh suspension of conidia on these discs. At a concentration of 12.5 conidia per mm³, 68% germinated on the surface of water agar alone, but only 38% of the conidia germinated when they were placed on water agar discs that had previously imbibed filtrate from a conidial suspension stored in corked test tubes.
Figure 11. Conidia of P. manshurica incubated 12 hours at 10 C on the surface of water agar after being stored in water in corked test tubes for 24 hours (A) at 5 C, (B) at 20 C
Figure 12. Conidia of *P. manshurica* incubated 12 hours at 10°C on the surface of water agar after being stored in water in corked test tubes for 24 hours at

(Upper left) 5°C, 400 conidia per mm$^3$
(Upper right) 20°C, 400 conidia per mm$^3$
(Middle left) 5°C, 200 conidia per mm$^3$
(Middle right) 20°C, 200 conidia per mm$^3$
(Lower left) 5°C, 100 conidia per mm$^3$
(Lower right) 20°C, 100 conidia per mm$^3$
for 12 days (Figure 13). No significant additional inhibitory effect was indicated by the filtrate obtained from spore suspensions stored in open beakers.

**Experiment 12. Retention of germination inhibitor in stored conidia**

Some of the conidia stored 12 days in beakers germinated on water agar. Although no self-inhibitor was detectable in spore-free filtrates of these conidia, the release of inhibitor associated with germinating conidia themselves could add to the inhibition of germination of fresh conidia. To test this hypothesis, the following experiment was performed: After the highest concentration of conidia had been incubated on the surface of water agar discs for 12 hours, the discs were turned over and drops of a dilution series of fresh conidia were incubated on the same discs for an additional 12 hours. Additional inhibitory effect was still associated with conidia stored in beakers (Figure 14). Germination inhibition obtained from a spore-free filtrate of a spore suspension held in corked test tubes 12 days (Figure 13) was about equal to the inhibition obtained from germinated spores which had been stored 12 days in an open beaker. These data give additional indication that the inhibitor was lost from a suspension of conidia exposed to the air, but was retained when the suspensions were stored in corked test tubes.
Figure 13. Percentage germination of the conidia of *P. manshurica* at various concentrations after incubation 12 hours at 10°C

(1) On the surface of water agar discs (control)
(2) On the surface of water agar discs which had imbibed the spore-free filtrate from a conidial suspension stored in open beakers 12 days at 5°C
(3) On the surface of water agar discs which had imbibed the spore-free filtrate from a conidial suspension stored in corked test tubes 12 days at 5°C. Experiment 11
Figure 14. Percentage germination of the conidia of *P. manshurica* at various concentrations after incubation 12 hours at 10°C

(1) On the surface of water agar (control)
(2) On the surface of water agar discs after conidia which had been stored 12 days in an open beaker at 5°C had incubated on the same discs for 12 hours at 10°C
(3) On the surface of water agar discs after conidia which had been stored 12 days in corked test tubes had incubated on the same discs for 12 hours at 10°C. Experiment 12
Germination of conidia after storage intact on leaves

Germination of conidia, in place, on sporulating surfaces of leaves was observed occasionally 24-72 hours after sporulation. This observation led to the hypothesis that the germination inhibitor was gradually being lost from spores left intact on leaves. Germination of a series of concentrations of freshly produced spores was compared with equal concentrations of conidia removed from leaves 72 hours after sporulation to test this hypothesis.

Comparison of the curves obtained when percentage germination was plotted against concentration of conidia (Figure 15) suggests that the inhibitor was lost from conidia left on the surface of leaves after sporulation. Conidia collected 72 hours after sporulation germinated 16% at the concentration of 100 conidia per mm$^3$. Only 2% of the conidia collected within a few hours after sporulation germinated. Comparison of germination at the concentration of 12.5 conidia per mm$^3$ indicates a decline in viability when conidia were left 72 hours on leaves in a moist chamber. If it were not for this decrease of viability, the differences in apparent inhibition would be greater still at the higher concentrations, thus emphasizing further that the inhibitor was lost from these conidia.
Figure 15. Percentage germination of the conidia of *P. manshurica* at various concentrations after incubation 12 hours on the surface of water agar discs at 10°C

(1) Conidia collected within 6 hours after sporulation
(2) Conidia collected 72 hours after sporulation. Experiment 13
Environmental factors such as temperature, humidity, moisture in the form of rain or dew, cloud cover, and wind all contribute to secondary spread of downy mildew in the field. Sporulation depends upon suitable temperature and a period of moisture in the form of dew on the leaves. Conidial discharge depends upon a decrease in humidity. Wind currents disperse and distribute the conidia after they are discharged. Conidia subsequently germinate on leaves only if moisture is present. The success of secondary infection, therefore, depends on the ability of the conidia to remain viable until conditions are favorable for their germination and infection.

A critical study of sporulation, conidial discharge and dispersal, longevity of conidia, and infection is a necessary prerequisite to a comprehensive understanding of secondary spread of \textit{P. manshurica} under field conditions. Several experiments were conducted to elucidate some of the interactions among environment, host, and pathogen in the laboratory, greenhouse, and field.

**Effects of light duration on sporulation and infection**

Comparison of infection and sporulation on greenhouse-grown soybeans in winter and summer months indicated that duration of daylight was involved in distinctive host-
parasite interactions. The expression of downy mildew symptoms was modified and mycelial development within the leaf was affected. The period of time during which sporulation occurred and the quantity of conidia produced per unit area of infected leaf surface also were influenced.

These observations prompted the design of an experiment to test the hypothesis that daily light duration influences mycelial growth of the fungus in leaf tissue and subsequent conidial production.

Experiment 14. The relative importance of light duration before or after inoculation of soybean seedlings with conidia

Pre-inoculation light treatments of one duration were alternated with post-inoculation light duration treatments of another duration. All plants which received pre-inoculation treatments of 6, 12, or 18 hours of light were given 12 hours of light after inoculation. Similarly, all plants which received post-inoculation treatments of 6, 12, or 18 hours were given 12 hours of light for the period before inoculation.

Symptoms were faintly visible first on leaves of plants which had received 18 hours of light both before and after inoculation. A few hours later, symptoms were faintly visible on all the other leaves of plants which had received other light duration treatments (Table 7). The infected areas of leaves exposed to six hours light before and after inocula-
Table 7. Ratings\(^a\) of downy mildew symptoms on unifoliate leaves of Illini soybean plants exposed daily to light for various lengths of time. Experiment 14

<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>Daily light exposure in hours</th>
<th>18</th>
<th>12</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre</td>
<td>Pre-post</td>
<td>Post</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>2.25(^b)</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>8</td>
<td></td>
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<tr>
<td>10</td>
<td></td>
<td>3.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>3.25</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>3.50</td>
<td>2.00</td>
<td>2.00</td>
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<tr>
<td>16</td>
<td></td>
<td>3.50</td>
<td>3.25</td>
<td>3.50</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>3.75</td>
<td>3.25</td>
<td>3.50</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>3.75</td>
<td>3.50</td>
<td>3.75</td>
</tr>
<tr>
<td>22</td>
<td></td>
<td>4.00</td>
<td>3.50</td>
<td>4.50</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>4.50</td>
<td>4.50</td>
<td>-</td>
</tr>
<tr>
<td>26</td>
<td></td>
<td>- (^c)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\) - no symptoms
2 - light green
3 - yellow green
4 - yellow
5 - orange to necrotic.

\(^b\) - Average of ratings on four replications.

\(^c\) - Unifoliate leaves necrotic.

...tion were the first to become chlorotic and were all necrotic by the sixteenth day. The infected areas of leaves which had been illuminated for 18 hours daily were still light green by the sixteenth day. They did not become necrotic until 24 days after inoculation.

Sporulation occurred, regardless of treatment, the sixth day after inoculation, although symptoms were only faintly visible that day. Conidial production was greatest on the
sixth day on the leaves of plants which had received 18 hours of light daily. Sporulation continued up to 24 days after inoculation on these plants. Conidial concentration was lowest on the leaves from plants which had been exposed to six hours of light both before and after inoculation. Conidia were not produced after the twelfth day when these plants were placed in a moist chamber for 12 hours (Table 8).

The infected areas of leaves given different light treatments were compared histologically. Fungus mycelium was abundant within the tissue of leaves exposed for the shorter

Table 8. Ratings\(^a\) of sporulation of *P. manshurica* on leaves of Illini soybean plants exposed daily to light for various lengths of time. Experiment 14

<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>Daily light exposure in hours</th>
<th>18</th>
<th>12</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre</td>
<td>Pre-</td>
<td>Post</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>4.00(^b)</td>
<td>4.25</td>
<td>4.75</td>
</tr>
<tr>
<td>8</td>
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<td>4.00</td>
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<td>3.75</td>
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<td>24</td>
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<td>2.50</td>
<td>2.50</td>
<td>-</td>
</tr>
<tr>
<td>26</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\)Based on scale ranging from 1 (no sporulation) to 5 (heavy sporulation).

\(^b\)Average of ratings on 4 replications.

\(^c\)Unifoliate leaves necrotic.
daily light periods. By comparison, leaves illuminated 18 hours daily contained much less intercellular mycelium. Initiation, development, and maturity of oospores of the fungus within the diseased leaf tissue were also highly responsive to the duration of light treatments. Oogonial initiation could not be observed six days after inoculation in the tissue of leaves exposed to 18 hours of light daily, but in leaves exposed six hours daily, numerous oogonia could be observed in various stages of development (Figure 16). Oospore development was related to the extent of chlorosis of diseased leaf tissue. There were relatively few oospores in light green areas of a particular leaf and these were in early stages of development. Oospores in adjacent chlorotic tissue were abundant and could be found in later stages of maturity. The oospores were mature in necrotic tissue (Figure 17).

The percentage area of leaves infected and yield of conidia per unit infected area were determined on the eighth day after inoculation for each of the various light duration treatments. The size of the leaf was significantly related to duration of light to which it was exposed daily (Figure 18). Leaves exposed to six hours of light before and after inoculation were significantly smaller than those obtained under longer daily light exposure. Conversely, leaves exposed for 18 hours both before and after inoculation were
Figure 16. Oospores of *P. manshurica* in the chlorotic tissue of diseased Illini soybean leaves ranging in stages of development from oogonial formation (upper left) through intermediate stages (upper right, lower left) to maturity (lower right)
Figure 17. Mature oospores of *P. manshurica* within the necrotic tissue of diseased *Illini* soybean leaves
Figure 18. Average area of unifoliate leaves of Illini soybean plants exposed daily to light for various lengths of time. Experiment 14

The difference between two treatment effects is judged significant at the 5% level if the means differ by 1.1 cm² or more.
significantly larger than leaves exposed to light for shorter daily periods.

The average percentage of leaf area infected was negatively related to the area of leaf and also negatively related to the duration of daily light treatment (Figure 19). Leaves which received six hours of light before and 12 hours after inoculation had a significantly greater percentage of leaf area infected than those receiving more light before inoculation. The leaves which received 18 hours of light both before and after inoculation had a significantly smaller percentage of leaf area infected than leaves which received any other treatment.

Yield of conidia from infected portions of the leaves was also significantly influenced by the daily duration of light (Figure 20). The largest yield of conidia was obtained from plants which received 18 hours of light both before and after inoculation. The lowest concentration of conidia was obtained from leaves which had been illuminated six hours daily. The concentration of conidia per unit area of infected leaf surface was, therefore, negatively related to the area occupied by mycelium in leaf tissue.

The size of infected areas in the resistant variety Woods Yellow did not change significantly with changes in light duration. The infected areas were not larger in leaves illuminated six hours daily than in leaves illuminated 18
Figure 19. Average percentage of unifoliolate leaf area of Illini soybean plants showing symptoms 8 days after inoculation with conidia of *P. manshurica* when seedlings were exposed daily to light for various lengths of time. Experiment 14

The difference between 2 treatment effects is judged significant at the 5% level if the means differ by 13.3% or more.
HOURS OF LIGHT RELATIVE TO INOCULATION

BEFORE + 12 AFTER
BEFORE AND AFTER
AFTER + 12 BEFORE

PERCENTAGE LEAF AREA INFECTED

HOURS OF LIGHT

6 12 18
Figure 20. Average number of conidia per mm$^2$ of infected unifoliate leaf surface 8 days after inoculation with conidia of *P. manshurica* when Illini soybean seedlings were exposed daily to light for various lengths of time. Experiment 14

The difference between two treatment effects is judged significant at the 5 percent level if the means differ by 240 or more conidia per mm$^2$. 
RELATIVE TO INOCULATION

HOURS OF LIGHT
BEFORE + 12 AFTER
BEFORE AND AFTER
AFTER + 12 BEFORE

NUMBER OF CONIDIA PER SQUARE MILLIMETER

HOURS OF LIGHT
6 12 18
The number of separate infection spots obtained on the leaves given 18 hours light daily was significantly greater than that obtained on leaves from plants grown under six hours of light daily (Figure 21).

**Experiment 15. Effects of soil moisture on sporulation and infection**

The results of experiment 14 indicated that physiological changes in soybean leaves conditioned by various light duration treatments had an effect on infection of leaves. Variable moisture supply to soil in which plants were grown also had an effect on infection. Therefore, the host-parasite interaction of amply-watered soybean seedlings as compared to seedlings grown under water stress was investigated.

One lot of Illini soybean plants was watered normally daily after the seedlings were inoculated with a uniform suspension of conidia. Another lot was watered only when plants showed signs of wilting. Infected areas of leaves of well-watered plants became chlorotic or necrotic within a three-week period and sporulation could not be induced when the leaves were placed in a moist chamber 12 hours. By contrast, the infected areas of leaves from plants grown under water stress were smaller in diameter and remained green for at least three weeks after inoculation. Abundant sporulation was obtained after placing these leaves in a moist chamber for 12 hours.
Figure 21. Infection loci per Woods Yellow soybean uni-foliate leaf 8 days after inoculation with conidia of *P. manshurica* when growing plants were exposed daily to light for various lengths of time. Experiment 14

The difference between two treatment effects is judged significant at the 5% level if the means differ by 3.7 or more infection loci per leaf
Experiment 16. Effects of temperature and moisture on sporulation

The initial requirement for successful secondary spread of *P. manshurica* is sporulation of the fungus. Two important environmental factors affect sporulation: temperature and moisture. The effect of the first of these factors was investigated under laboratory conditions.

Sporulation of *P. manshurica* on infected Illinois soybean leaves was determined at 10, 15, 20, 25, and 30 °C. Leaves were placed in plastic box moist chambers and left at the respective temperatures for 12 hours. The leaves were removed and the conidia were washed off in water. No sporulation occurred when leaves were placed in a moist chamber for 12 hours at 10 or 30 °C, but abundant conidia were produced at 15, 20, and 25 °C (Table 9).

The significance of the complete absence of sporulation of the fungus at temperatures of 10 °C or below, or 30 °C or above, is reflected in the adaptation of this organism to

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Number conidia $\times 10^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>1.35</td>
</tr>
<tr>
<td>20</td>
<td>1.46</td>
</tr>
<tr>
<td>25</td>
<td>0.77</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
</tr>
</tbody>
</table>

*Each number is based on the average of 5 replications of 4 leaves each.*
infection of soybeans within the normal night temperature range encountered during the growing season when soybean plants are most susceptible. Night temperatures were never as low as 10°C, nor as high as 30°C during an observed period of rapid secondary spread of downy mildew in the field. Secondary spread was not prevented, therefore, by the factor of adverse temperature on sporulation during this period.

Sporulation of *P. manshurica* was never observed unaccompanied by visible moisture on the surface of infected leaves. Sporulation from infected areas of leaves was readily induced by spraying plants with water from an atomizer and placing them in closed plastic bags or in tight containers which also held sphagnum moss saturated with moisture. Evidence of fresh sporulation in the field was critically dependent upon the presence of free moisture, particularly on the lower sides of the leaves.

**The effect of changes in relative humidity on conidial discharge**

Mass conidial discharge from the surface of leaves was observed immediately following the removal of infected plants from a moist chamber. In still air of low relative humidity, the conidia could be seen to float away in clouds from the sporulating surfaces of the leaves.

The exact mechanism of spore discharge was observed under the microscope. Leaves bearing freshly-formed conidio-
Figure 22. (A) Conidiophores of *P. manshurica* intact on the surface of a soybean leaf, after being exposed to a dry atmosphere

(B) Dry conidia of *P. manshurica* after being discharged from conidiophores
the leaf after their removal from the conidiophores were collapsed (Figure 22B). They immediately regained their round, turgid appearance when placed in water.

**Longevity of conidia under laboratory conditions**

Several experiments were performed to determine longevity of conidia when they were held under various conditions in the laboratory. In some of the investigations conidia were stored in water at various temperatures. In others, the conidia were left intact on the leaves and stored under various conditions of temperature and humidity. In all of the experiments, ability to cause infection of soybean leaves after the storage periods was the criterion of viability. No attempts were made to determine conidial germinability or to estimate viability on a quantitative basis. Care was taken to insure that equivalent concentrations of conidia and uniform inoculation procedures were used each time a lot of conidia was assessed for viability.

**Experiment 17. Differences between longevity of conidia stored aerobically or anaerobically in water** Conidia at a concentration of 400 per mm³ were stored in individual test tubes of distilled water at 1 and 10 °C, and in freshly boiled, distilled water covered with 2 ml of mineral oil at 1 °C. Mineral oil was used to prevent oxygen from dissolving in the water during the time conidia were in storage.
Storage in distilled water or in boiled water under mineral oil had no effect on the longevity of conidia (Table 10). The effect of temperature was noticeable, however. Conidia stored at 10 C did not remain viable to any appreciable extent for more than 11 days. Some of the conidia remained viable when stored 19 days in water at 1 C.

Table 10. Percentage leaf area of Illini soybean seedlings infected by P. manshurica when the leaves were inoculated with conidia stored for various lengths of time in distilled water at 1 C, 10 C, or in boiled, distilled water covered with mineral oil at 1 C. Experiment 17

<table>
<thead>
<tr>
<th>Days of storage</th>
<th>Conidia stored under oil</th>
<th>In water</th>
<th>In water</th>
<th>In water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 C</td>
<td>1 C</td>
<td>10 C</td>
</tr>
<tr>
<td>0</td>
<td>90^a</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>80</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td>90</td>
<td>90</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>11</td>
<td>90</td>
<td>80</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>19</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

^aEach figure represents the average infected leaf area on four replications of four plants each.

Experiment 18. The influence of temperature and humidity on longevity of conidia left intact on infected leaves after sporulation. Infected leaves of Illini soybeans were detached after sporulation had occurred. They were placed in desiccators containing water, saturated sodium chloride solution, or dry calcium chloride, producing relative humidities
of 100, 80, and 0%, respectively. The desiccators were placed in temperature-controlled chambers at -14, 1, and 20 C. The relative amount of infection declined only after 14 days when Illini seedlings were inoculated with conidia stored on leaves at -14 and 1 C at any of the three relative humidities. At 20 C, however, viability began to decline after the fifth day of storage at all three humidities. Results of other similar experiments also indicated that variation of humidity in storage was not as important to longevity of conidia as variation in temperature.

**Experiment 19. Longevity of conidia sprayed on plants exposed to normal summer conditions**

Illini soybean seedlings were inoculated with a uniform suspension of conidia at 9:30 AM. Four pots of the plants were placed in a moist chamber immediately after inoculation and the others were placed out of doors in direct sunlight in a location unsheltered from the wind. At intervals during the following 28 hours, four of these pots were placed in a moist chamber. The maximum temperature recorded outdoors on the day of the experiment was 30 C and the minimum relative humidity was 58%. The wind was variable with velocities averaging 300 feet per minute at the beginning of the experimental period and over 600 feet per minute by 5 PM. With wind of this velocity, the plants were kept in constant motion. Gusts of wind often agitated the leaves violently.
The number of infection loci obtained on the leaves plotted against time of exposure of plants to the weather resulted in the curve shown in Figure 23. A total of 590 infection loci were counted on the leaves of plants placed in the moist chamber immediately after inoculation, but only 17 infection loci were obtained on leaves of plants that were placed in a moist chamber 24 hours later. This represented a 97% decrease in potentially viable conidia.

The most significant aspect of these results lies in the fact that approximately 13% of the conidia were viable by 8:00 PM of the first day. Survival of conidia on leaves throughout the adverse weather conditions of the day is important to the success of secondary spread of downy mildew in the field. Favorable conditions for germination of conidia were observed often during the early evening hours. Sufficient dew was formed on the leaves so that conidia lying there from the time they were discharged in the morning could germinate and cause infection.

Experiment 20. Secondary spread under field conditions

Five general principles were developed from the results of a field experiment designed to study sporulation, conidial discharge, longevity, and infection in response to environmental conditions. They are presented in the form of statements and illustrated by portions of the data gathered over a 17-day period during August, 1959. General observations
Figure 23. Number of infection loci on Illini soybean unifoliate leaves incubated immediately and at various intervals of time after inoculation on 15 August, 1960. Experiment 19
of environmental conditions encountered on the days selected for consideration are presented in Table 11.

First principle: Heavy plant infection on any particular day is dependent on a period of at least ten hours of recorded dew the preceding night. Without sufficient dew deposition, fresh sporulation on leaves of the field plants could not be observed to any appreciable extent. Consequently, little or no infection was obtained on the test plants. Comparison of the data obtained on 8 and 9 August (Figure 24) illustrates this principle. Maximum humidity of 85% was reached at 6:00 AM and dew was not recorded on the morning of 8 August. Few infection loci were obtained on test plants placed in the field that day. The next day, however, was preceded by 13 hours of dew. The relative humidity was 100% for 9½ hours. Abundant sporulation was observed on the infected areas of leaves of field plants in the morning and a large number of infection loci were obtained on the test plants that were in the field that day.

Second principle: The period of most abundant conidial discharge occurs concurrently with the period of rapid drying of field plants following a period of heavy dew. The data obtained on 13 August (Figure 25) illustrate this principle. The largest number of infection loci per hour was obtained between 8:15 and 10:30 AM. During this period, the relative humidity rapidly declined to 50% and the leaves were dried
Figure 24. Influence of relative humidity and duration of moisture on natural infection of Hawkeye soybean leaves by *P. manshurica*. Plants placed in the field in the evening and removed the following day at the hours indicated. Experiment 20.
Figure 25. Influence of relative humidity and duration of moisture on natural infection of Hawkeye soybean leaves by P. manshurica. Plants placed in the field for the intervals indicated and then removed and placed in moist chambers. Experiment 20
Table 11. Observations of environmental conditions during August, 1959. Experiment 20

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Moisture</th>
<th>Wind velocity ft./min.</th>
<th>Cloud cover</th>
<th>Sporulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/8/59</td>
<td>4:05 AM</td>
<td>None</td>
<td>100</td>
<td>Overcast</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>6:00 AM</td>
<td>None</td>
<td>120</td>
<td>Heavy overcast</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8:00 AM</td>
<td>None</td>
<td>280</td>
<td>Heavy overcast</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10:40 AM</td>
<td>None</td>
<td>250</td>
<td>Heavy overcast</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7:00 PM</td>
<td>None</td>
<td>Calm</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td>8/9/59</td>
<td>4:05 AM</td>
<td>Heavy dew</td>
<td>Calm</td>
<td>Clear</td>
<td>Abundant</td>
</tr>
<tr>
<td></td>
<td>6:00 AM</td>
<td>Very heavy dew</td>
<td>Calm</td>
<td>Light fog</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8:30 AM</td>
<td>Very heavy dew</td>
<td>50-100</td>
<td>Clearing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11:25 AM</td>
<td>None</td>
<td>100-160</td>
<td>Partly cloudy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:00 PM</td>
<td>None</td>
<td>100-160</td>
<td>Partly cloudy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10:00 PM</td>
<td>None</td>
<td>Calm</td>
<td>Cloudy</td>
<td></td>
</tr>
<tr>
<td>8/12/59</td>
<td>6:30 AM</td>
<td>None</td>
<td>280-400</td>
<td>Partly cloudy</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>8:35 AM</td>
<td>None</td>
<td>500-750</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11:30 AM</td>
<td>None</td>
<td>500-750</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:10 PM</td>
<td>None</td>
<td>300-500</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4:35 PM</td>
<td>None</td>
<td>180-380</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Time</td>
<td>Moisture</td>
<td>Wind velocity ft./min.</td>
<td>Cloud cover</td>
<td>Sporulation</td>
</tr>
<tr>
<td>-----------</td>
<td>--------</td>
<td>------------------</td>
<td>------------------------</td>
<td>----------------</td>
<td>-------------</td>
</tr>
<tr>
<td></td>
<td>8:30 PM</td>
<td>Faintly visible dew</td>
<td>Calm</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td>8/13/59</td>
<td>6:00 AM</td>
<td>Heavy dew</td>
<td>Calm</td>
<td>Partly cloudy</td>
<td>Abundant</td>
</tr>
<tr>
<td>8/13/59</td>
<td>8:15 AM</td>
<td>Upper leaves drying</td>
<td>Rustles leaves</td>
<td>Hazy</td>
<td></td>
</tr>
<tr>
<td>8/13/59</td>
<td>10:30 AM</td>
<td>None</td>
<td>600-700</td>
<td>Hazy</td>
<td></td>
</tr>
<tr>
<td>8/13/59</td>
<td>1:25 PM</td>
<td>None</td>
<td>650-1150</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td>8/13/59</td>
<td>4:05 PM</td>
<td>None</td>
<td>650-1150</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td>8/13/59</td>
<td>8:00 PM</td>
<td>None</td>
<td>Calm</td>
<td>Cloudy</td>
<td></td>
</tr>
<tr>
<td>8/14/59</td>
<td>6:05 AM</td>
<td>Very light dew</td>
<td>Calm</td>
<td>Cloudy</td>
<td>None</td>
</tr>
<tr>
<td>8/14/59</td>
<td>8:15 AM</td>
<td>None</td>
<td>Calm</td>
<td>Hazy</td>
<td></td>
</tr>
<tr>
<td>8/14/59</td>
<td>10:50 AM</td>
<td>None</td>
<td>100-250</td>
<td>Hazy</td>
<td></td>
</tr>
<tr>
<td>8/14/59</td>
<td>1:50 PM</td>
<td>None</td>
<td>120-320</td>
<td>Hazy</td>
<td></td>
</tr>
<tr>
<td>8/14/59</td>
<td>4:25 PM</td>
<td>Rain</td>
<td>Calm</td>
<td>Overcast</td>
<td></td>
</tr>
<tr>
<td>8/14/59</td>
<td>6:25 PM</td>
<td>Rain</td>
<td>Calm</td>
<td>Overcast</td>
<td></td>
</tr>
<tr>
<td>8/15/59</td>
<td>9:00 AM</td>
<td>Rain</td>
<td>Calm</td>
<td>Overcast</td>
<td>Very little fresh sporulation on a few leaves</td>
</tr>
<tr>
<td>8/15/59</td>
<td>12:00 PM</td>
<td>Dry</td>
<td>Calm</td>
<td>Overcast</td>
<td></td>
</tr>
<tr>
<td>8/15/59</td>
<td>3:15 PM</td>
<td>Dry</td>
<td>100-300</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td>8/15/59</td>
<td>7:05 PM</td>
<td>Dry</td>
<td>Calm</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Time</td>
<td>Moisture</td>
<td>Wind velocity ft./min.</td>
<td>Cloud cover</td>
<td>Sporulation</td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
<td>--------------------------------</td>
<td>------------------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>8/17/59</td>
<td>6:00 AM</td>
<td>Very heavy dew</td>
<td>Calm</td>
<td>Clear</td>
<td>Abundant</td>
</tr>
<tr>
<td></td>
<td>8:15 AM</td>
<td>Very heavy dew</td>
<td>100</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10:45 AM</td>
<td>Drying, leaves in shade still wet with dew</td>
<td>Calm</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:50 PM</td>
<td>Dry</td>
<td>100</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5:00 PM</td>
<td>Dry</td>
<td>100</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8:30 PM</td>
<td>Heavy dew</td>
<td>Calm</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td>8/18/59</td>
<td>6:15 AM</td>
<td>Very heavy dew</td>
<td>Calm</td>
<td>Clear</td>
<td>Abundant</td>
</tr>
<tr>
<td></td>
<td>8:00 AM</td>
<td>Very heavy dew</td>
<td>300-360</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10:30 AM</td>
<td>Drying, leaves in shade still wet with dew</td>
<td>300-400</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:50 PM</td>
<td>Dry</td>
<td>400-500</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5:00 PM</td>
<td>Dry</td>
<td>400-600</td>
<td>Clear</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8:00 PM</td>
<td>Dry</td>
<td>Calm</td>
<td>Clear</td>
<td></td>
</tr>
</tbody>
</table>
rapidly. After 10:30 AM, the number of infection loci obtained on the leaves gradually declined until, during the period 4:00 to 8:00 PM, only 12 infection loci per hour were obtained. These results substantiate the conclusion drawn from observations of the mechanism of conidia discharge—i.e. conidia are actively and forcibly removed from the conidiophores following lowered humidity.

Third principle: Freshly sporulated conidia surviving daytime conditions of temperature and humidity can germinate the following night and cause infection if the conditions for germination are favorable. This principle is illustrated by the data obtained on 13 and 17 August (Figure 26). The 12½ hour period of dew recorded from 7:00 PM on 12 August to 7:30 AM on 13 August was favorable for abundant sporulation during the morning of 13 August. Numerous infection loci were obtained on the test plants exposed that day if they were placed in plastic bag moist chambers the following morning when taken out of the field, but very few infection loci were obtained if the test plants were not placed in plastic bags. The dew period recorded for 14 August did not exceed eight hours and the relative humidity was 100% only for a short time at 6:00 AM. Apparently dew was not heavy enough, nor did it persist for a sufficient length of time on the test plants to permit germination and infection by conidia that had fallen on them during the preceding day. During
Figure 26. Influence of relative humidity and duration of moisture on natural infection of Hawkeye soybean leaves by *P. manshurica*. Plants placed in the field at the hours indicated and all removed from the field the following morning. Experiment 20
the night of 17 August and the morning following, however. 13\frac{1}{2} hours of dew were recorded. This period of moisture was favorable for germination of conidia on the leaves of the test plants. Therefore, similar curves were obtained for the number of infection loci on plants whether they were placed in moist chambers or not.

The third principle is also illustrated by the data obtained on 13 August (Figures 25 and 27). Humidity reached a low of 32\% at 2:00 PM and the temperature maximum was 30 C. Plants placed in plastic bags and taken from the field at 8:00 PM that day developed 535 infection loci. An average of only 12 infection loci per hour were obtained on plants remaining in the field during the period 4:00 to 8:00 PM (Figure 27). The 535 infection loci, therefore, represented conidia which accumulated on the leaves throughout the day. These conidia survived the daytime weather conditions, germinated, and caused infection after the plants were placed in moist chambers.

Although plants placed in the field in the evening usually did not become infected unless they were placed in plastic-bag moist chambers when they are taken out of the field during the following day, one notable exception occurred on 17 August (Figure 28). About the same number of infection loci were obtained on uncovered as on covered plants. Records of dew deposition or humidity do not indicate
Figure 27. Influence of relative humidity and duration of moisture on natural infection of Hawkeye soybean leaves by P. manshurica. Plants placed in the field in the evening and removed the following day at the hours indicated. Experiment 20
Figure 28. Influence of relative humidity and duration of moisture on natural infection of Hawkeye soybean leaves by *P. manshurica*. Plants placed in the field in the evening and removed the following day at the hours indicated. Experiment 20
PLANTS REMOVED FROM FIELD AND PLACED IN MOIST CHAMBER FOR 24 HOURS

PLANTS NOT PLACED IN CHAMBER

NUMBER OF INFECTION LOCI TEN DAYS AFTER EXPOSURE TO FIELD INOCULUM

DURATION OF MOISTURE

RELATIVE HUMIDITY

AUG 16, 1959  AUG 17, 1959  AUG 18, 1959

TIME AND DATE
a logical reason for these results. A study of some of the other environmental conditions, however, provides a reasonable explanation. The night of 15 August was favorable for sporulation. Relative humidity was over 90% and 14 hours of dew were recorded. The day of 16 August was cool (not over 23°C) and humid (not less than 75% relative humidity) and probably not ideal for rapid conidial discharge and dispersal. The leaves did not dry off rapidly during the day. Leaves were wet with dew again by 8:30 PM when the test plants were placed in the field. It is probable that viable conidia were still present in abundance in the evening of 16 August when the test plants were placed in the field. Those that fell on the plants germinated and caused infection. The morning of 17 August, in contrast with the preceding day, was more favorable for spore discharge and dispersal. The relative humidity dropped to 32% during the day. Dew was heavy on leaves by 8:30 PM again that evening, thus accounting for the presence of some infection loci on uncovered plants taken in from the field at various times on 18 August.

Fourth principle: Viable conidia are present in the air on days following nights unfavorable for sporulation. For example, 36 infection loci were obtained on the leaves of plants taken out of the field at 4:00 AM on 8 August (Figure 24). Despite this fact, there was no recorded dew and no visible fresh sporulation for 48 hours preceding 8 August.
A few infection spots were found on test plants placed in the field after two consecutive days of unfavorable conditions for sporulation. This indicated that some conidia remained viable for more than 48 hours in the field.

Fifth principle: Long periods of moisture deposition or of high relative humidity will not necessarily induce heavy sporulation. The dew recorder indicated 20 hours of continuous moisture on 14 and 15 August and the humidity was over 90% most of the period (Figure 29). This record, however, represented moisture from rain. The number of infection loci counted on plants that had been placed in the field on 15 August averaged only 40 per hour when the plants were in the field from 9:00 to 12:00 AM, and only 25 per hour later in the day when foliage was drying. Although tops of the leaves in the field were wet constantly during this 20 hour period, the lower surfaces were comparatively dry. Very little fresh sporulation could be detected during this long period of moisture.
Figure 29. Influence of relative humidity and duration of moisture on natural infection of Hawkeye soybean leaves by *P. manshurica*. Plants placed in the field for the intervals indicated and then removed and placed in moist chambers. Experiment 20
The biological functions of the germination inhibitor are prevention of premature germination, extension of the germination period, and suppression of germination of other species of fungal spores in the immediate vicinity of the species possessing the inhibitor. If conidia were to germinate in place after their formation, the chance for dispersal of the species over wide areas would be reduced. Although conidia are kept away from the moist surface of a leaf by conidiophores, the additional factor of a self-inhibitor serves also to prevent germination before the conidia become adequately disseminated.

Evidence that a germination inhibitor is present in conidia, uredospores, and apparently present in infected portions of leaves (Wilson 1958), would lead one to suspect that it may also be present in oospores of *P. manshurica*. The reason for erratic results of systemic infection experiments and complete lack of success in germinating oospores might have been the presence of a germination inhibitor which prevented their germination while associated in high concentrations. The relative success of using talc mixed with oospores for inoculation of seed may have been due primarily to the dilution effect. The highest percentage of systemic infec-
tion was obtained when activated charcoal was used as a diluent of oospores. The charcoal may have been active in adsorption of inhibitor. Crosier (1934) found that by adding bentonite clay or other colloidal substances to suspensions of sporangia of Phytophthora infestans, increases in germination up to 231% were obtained.

Many workers have found that oospores of other members of the Peronosporaceae germinate erratically. Success in germinating them has usually been associated with two factors: aeration and moisture. Aside from the obvious necessity of oxygen for respiration and water for enzyme activity in the germination process, one or both of these factors may have been required for reducing the concentration of a germination inhibitor present in the oospores. Repeated washing of oospores or extended periods of aeration of moist oospores to deplete the inhibitor may be worthy of trial to obtain germination and improve the percentage of systemic infection by seed inoculation.

Discovery of the chemical substances controlling inhibition and the exact nature of the controlling mechanism may have far reaching implications in designing specific fungitoxicants and chemotheropentants.
Influence of Environmental Factors on 
host-pathogen interactions

An understanding of disease development in the field is 
dependent upon the elucidation of the specific influence 
environmental factors have on development of the pathogen 
in host tissue, sporulation potentials, conidial dispersal, 
and subsequent reinfection. Environmental factors influence 
host-pathogen interactions directly and indirectly. Some of 
the indirect effects on the fungus are conditioned by light 
and moisture. They both affect growth of the host which in 
turn indirectly affects both vegetative and reproductive 
phases of development of the pathogen.

The effect of light on the host-pathogen interaction 
may be purely physical or it may be entirely biochemical. 
It may be a combination of these. Physical aspects of the 
interaction may be a thickening or strengthening of cell 
walls, or reduction in size of pit openings so that the fungus 
haustoria have physical difficulty in penetrating the walls 
of the cells they parasitize. On the other hand, the reaction 
may be biochemical. Products formed by the plant cell under 
longer duration of light or under moisture stress may be 
fungitoxic, or specific metabolites required by the fungus 
may be supplied in different proportions so mycelial develop­
ment never becomes extensive. The propensity for sporulation 
may be stimulated by a starvation process under long light
duration. This may be a compensatory reaction by the fungus. On the other hand, specific metabolites available to the fungus when plants are grown under longer light durations may be stimulatory to the differentiation reaction of sporulation.

The stimulus for oospore formation appears to be directly associated with the onset of chlorosis of leaf tissue. This seems to indicate a starvation reaction. It would be interesting to know what materials are being depleted out of proportion to others in leaf cells.

The diurnal cycle of dissemination indicates that conidia are discharged as the conidiophores shrivel in the morning during a period of rapidly drying dew. Although spore discharge rapidly diminishes after a short maximum peak in the morning, a much higher proportion of the conidia which become entrapped on the leaf surface remain viable than was once thought.

Spread of downy mildew in the field is not dependent upon sporulation alone, but vitally dependent on longevity of the conidia after their dissemination until they germinate on susceptible leaves. Many workers have concluded that conidia of other downy mildews are short-lived during the day and must, therefore, fall on susceptible leaves and germinate in the morning while there is still dew in order to reinfect the host. This conclusion is based on insuf-
icient evidence. Evidence obtained from laboratory studies indicates conidia remain viable for at least 5 days at 20°C when they are stored at 0 or 80% relative humidity. These findings agreed with the evidence obtained from field experiments—i.e. some conidia remain viable for at least 48 hours after fresh sporulation may have occurred. Secondary infection and disease build-up is, therefore, dependent not only upon conditions conducive to sporulation and conidial discharge, but also upon conditions which will permit conidial germination. It has been shown that secondary infection does not require periods of continuous moisture; rather, successive nights with heavy dews are sufficient. The conclusions drawn from these field studies are of fundamental importance in forecasting epiphytotics of the disease. For example, on a day with rapidly declining humidity following a night of heavy dew, spore dispersal will occur in abundance and many of the spores that fall on the plants will survive the daytime weather conditions to germinate on susceptible leaves the next night if another heavy dew occurs. Moisture in the form of rain is not significant if it follows a period unfavorable for sporulation. Secondary infection is favored only when rain follows within a few hours after conditions favorable for sporulation and conidial dispersal have occurred. Rain definitely does not favor sporulation. Although the tops of the leaves are wet during a rain, the lower sides where
sporulation occurs remain comparatively dry.

The dew record appears to be of more importance in determining probability of heavy sporulation than humidity records. During some nights, the relative humidity did not exceed 90% for more than eight hours, but at the same time, the dew recorder indicated a deposit of dew 12 hours in duration. In addition, it appeared that the amount of dew on leaves significantly influenced the amount of inoculum produced. Sporulation was often observed to be light following a night of light dew deposit, although the period of dew recorded should have been long enough to permit heavy sporulation.

An instrument which would record the amount of dew as well as the persistence and length of dew period would be invaluable for predicting the probability of heavy sporulation.
Systemic infection of soybeans by *P. manshurica* was obtained by planting oospore encrusted seeds and by inoculating seeds with oospores from encrusted seeds or diseased leaf material. Methods of inoculation which assured intimate contact between embryo and inoculum resulted in a higher percentage of systemically infected seedlings than occurred when inoculum was deposited externally on intact seeds. A higher percentage of systemic infection resulted when inoculated seeds were germinated at 15 and 20 C than at 25 and 30 C. The highest percentage of seedlings became systemically infected when activated charcoal mixed with oospores was used as inoculum.

Conidia remained viable at least 19 days stored aerobically or anaerobically in water at 1 C and at least 14 days stored intact after sporulation on leaves at -14 and 1 C.

Percentage germination of conidia was inversely proportional to their concentration on the surface of water agar. The adverse effect of high concentration on germination was due to an inhibitor associated with the conidia. The inhibitor diffused readily in water and was lost from aerated suspension, but was retained in suspensions stored anaerobically.

Size of leaves, number of conidia produced per unit area of infected leaf surface, and period of days during which
sporulation occurred were directly proportional to duration of daily exposure of plants to light. Conversely, the percentage of leaf area infected was inversely proportional to duration of light. Oospores developed in infected leaf tissue simultaneously with the onset of chlorosis. They developed earlier and in greater numbers in leaves given short daily exposures to light than in leaves given long daily exposures.

Duration of dew was the environmental factor most influential in determining success of secondary infection of downy mildew in the field. A ten-hour period of heavy dew was favorable for heavy sporulation. Sporulation did not occur after prolonged moisture deposition by rain. High humidity records were not as valuable as dew records for prediction of sporulation potentials.

Peak conidial discharge occurred concurrently with rapid drying of field plants following a period of heavy dew. Conidia discharged during the morning hours survived conditions of high temperature and low humidity and caused infection the following night provided conditions for conidial germination were favorable.
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