# EFFECTS OF VARIATION IN WATER POTENTIAL ON THE VIABILITY AND BEHAVIOUR OF CONIDIA OF *PERONOSPORA TABACINA* ADAM

By C. J. SHEPHERD,* PENELOPE SIMPSON,* and ANNETTE SMITH*

[*Manuscript received January* 29, 1970]

*Abstract*

A method is described for the collection of dry conidia of *P. tabacina*.

Conidia were shown to lose or gain water according to the ambient humidity. When stored at 91·4% R.H., 50% of conidia were swollen, while all were shrunken at 88% R.H. and all swollen at 94% R.H. Average weight per conidium increased from 1·04 ng at 0% R.H. to 2·65 ng at 99% R.H.

Immersion of conidia in sucrose solutions caused shrinkage and prevented germination. When transferred to water, germination occurred, indicating that reduction of germination in sucrose solutions was due to reduced water potential and not to loss of viability.

Conidial viability was affected by temperature (5–25°C) and relative humidity, viability decreasing most rapidly at the higher temperatures and humidities. When conidia were allowed to start germination and then dried, they remained capable of completing germination, but their viability gradually fell to zero, the rate of fall being most rapid at high temperatures and humidities.

At low relative humidities conidia were considerably more resistant to inactivation by ultraviolet irradiation than were conidia in water. Low humidities also decreased the effect of high temperatures on conidial viability.

The rate of sedimentation of conidia in still air was lower at low relative humidities (0·742 cm/sec at 34% R.H.) than at high humidities (0·953 cm/sec at 79% R.H.).

The epidemiological significance of these findings is discussed.

## I. INTRODUCTION

Gregory (1961) has noted that, in air, spores gain or lose water rapidly. A number of studies (Buller 1909, 1922; Falck 1927; Weinhold 1955) have been made on the sedimentation of spores in still air and the effects of desiccation on their rates of fall, but fewer authors have investigated the effects of desiccation on other properties of spores. Grindle and Good (1961) and Good and Zathurecsky (1967) have studied the effects of drying on the viability of germinating and germinated conidia of several fungi. Von Stille (1965) demonstrated the influence of osmotic pressure on the germination of sporangia of *Phytophthora infestans*, an increased osmotic pressure being found to favour direct germination, whereas Crozier (1934) showed that conidia of *P. infestans* survived for less than 8 hr at 90% R.H. and for only a few hours at lower humidities.

* Division of Plant Industry, CSIRO, P.O. Box 109, Canberra City, A.C.T. 2601.

In 1932, Angell and Hill reported that the longevity of conidia of *Peronospora tabacina* Adam was affected by changes in humidity and storage temperature but interpretation of their results was difficult owing to the inadequate germination techniques available at that time. Subsequently, Hill (1962) investigated the effects of a range of humidities on the longevity of conidia of *P. tabacina* stored on leaf disks and showed that the conidia were capable of surviving for some weeks under suitable conditions. More recently, Bromfield and Schmitt (1967) have shown that conidia of this fungus will remain viable for 25 months under cryogenic storage. Other studies (Cruickshank 1961; Shepherd 1962; Hill 1966) have been concerned with the properties of *P. tabacina* conidia in aqueous suspension only.

This study is concerned with the delineation of the factors influencing conidial viability and behaviour during the dissemination period and we have examined the effects of varying water potential (Kramer, Knipling, and Miller 1966) on the shrinking and swelling, the viability, the germination capacity, the sensitivity to ultraviolet irradiation, and the rate of fall in still air of conidia of *P. tabacina*.

## II. Materials and General Methods

The production of conidia of *P. tabacina* has been described by Shepherd (1962). At the start of these experiments, conidia were harvested dry by the cyclone collector method of Tervet *et al.* (1951), or by an apparatus similar to that described by Woodbury, Macko, and Stahmann (1957). However, the following technique was more satisfactory for the rapid collection of large numbers of dry conidia and was used for collecting the bulk of the spores used in these studies. Sporulating leaves were placed, lower side uppermost, on the laboratory bench until dry. Conidia were then removed by suction, using the basal portion of a Millipore filter holder covered by a Millipore filter disk (1·2 μm pore size and 2·5 cm diam.) and held a few millimetres above the leaf surface. Conidiophores and debris were removed by sieving through fine nylon gauze and conidia were either used immediately or stored at 4°C in a desiccator over anhydrous calcium chloride; in the latter case, they were used as soon as convenient, or discarded after 7 days storage.

Data on the relationship of concentration to osmotic pressure for sucrose solutions were kindly provided by B. Millar of the Pye Field Environment Laboratory, CSIRO. Information on the constitution of glycerine–water, sodium chloride–water, and sulphuric acid–water systems for the maintenance of a series of standard relative humidities was obtained from Middleton and Spilhaus (1953).

## III. Experimental and Results

### (*a*) *Effects of Ambient Humidity on the Water Content of Conidia*

In dry air, conidia of *P. tabacina* present a shrunken appearance, with invagination of irregular areas of wall, although the ellipsoid shape of the swollen conidia in water is still apparent. Because of their high refractility and irregular shape, accurate measurement of their dimensions is not possible. Thus, the degree of shrinking or swelling cannot be ascertained by measuring conidial dimensions. In the experiments recorded below, those conidia showing invagination of the wall are classed as "shrunken", while those that show no such invagination are classed as "swollen".

The effects of various humidities on the swelling of dry conidia were examined by dusting conidia on to microscope coverslips suspended in small bottles containing appropriate solutions for the control of humidity (see Section II). After 48 hr

incubation at 20°C the spores were examined *in situ*, without opening the humidity control vessels, under the microscope set up in a 20°C constant-temperature room. Figure 1 shows the relationship between the "bar" equivalents of the relative humidities used and the numbers of conidia swollen in two separate experiments. The relative humidity (91·4%) giving 50% swollen spores indicates the conidia to have an equivalent osmotic potential of approximately −121·6 bars. No germination of spores was observed following incubation under the above conditions.

Fig. 1

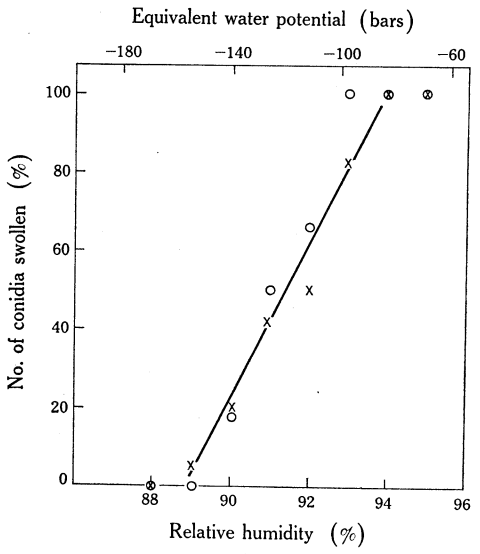


Fig. 2

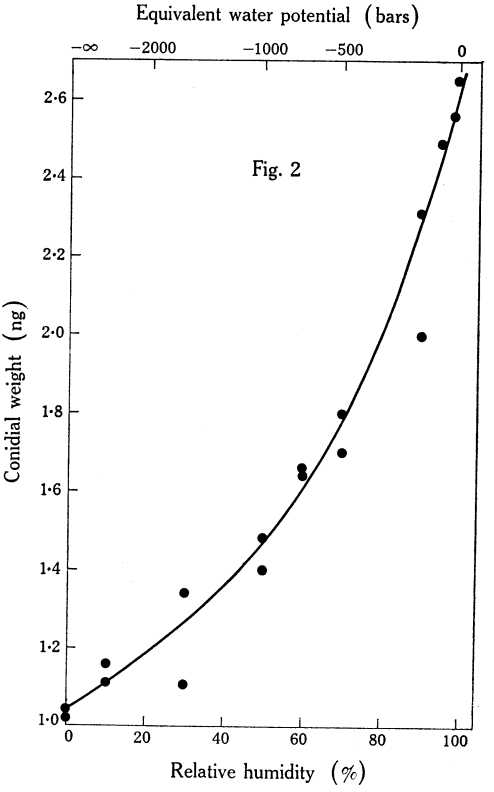


Fig. 1.—Effect of exposure to a range of relative humidities on swelling of dry conidia. ○ Experiment 1. × Experiment 2.

Fig. 2.—Effect of ambient humidity on conidial weight.

For investigation of the water content of conidia kept at various humidities, approximately 100 mg of spores were weighed into tared, stoppered, weighing bottles. These were placed in wide-necked bottles containing appropriate solutions for the control of humidity, a sliding wire being passed through the stopper of the humidity control vessel, so that the stopper of the weighing bottle could be removed, or replaced, *in situ* without opening the humidity control vessel. In order to minimize temperature variations during the course of the experiment (thus ensuring minimum changes in relative humidity) the humidity control vessels were immersed in a large tank of water kept in a constant-temperature room at 20°C.

Preliminary experiments indicated that equilibration between spores and vapour phase was complete in 12–24 hr but to allow a sufficient margin of safety conidia were left for 48 hr at the various humidities before being reweighed. Following this, conidia were oven-dried at 105°C for 17 hr and again reweighed. Conidial weight was estimated by the method of Weinhold (1955).

The relationship between the "bar" equivalents of ambient humidity and conidial wet weight is shown in Figure 2. Over the same range of humidities, Figure 2 shows that total conidial weight increases approximately 2·5 times.

### (*b*) *Swelling and Shrinkage of Conidia in Sucrose Solutions of Varying Water Potential and the Effects of these Solutions on Conidial Viability*

Dry conidia were suspended in sucrose solutions of differing molalities and in water to give a concentration of $5\times10^3$/ml. The numbers of swollen conidia were determined at intervals of 3, 10, 20, 30, and 40 min after immersion, by means of a Wild microscope fitted with a projection head and using a magnification of ×450. Fifty spores were examined from each of two samples and the pooled results of three such experiments are shown in Figure 3. At sucrose molalities between 0–5, there

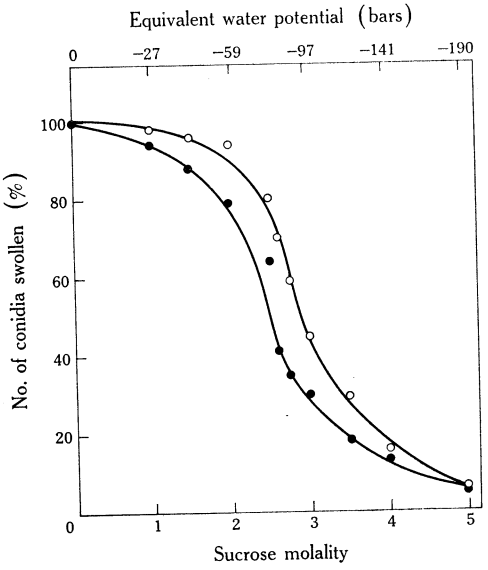


Fig. 3.—Effect of immersion in sucrose solutions of differing molality on swelling of dry conidia.
● After immersion for 3 min.
○ After immersion for 40 min.

was some increase in the numbers of swollen conidia during the first 20–30 min of observation, but there was no further increase after this period. The increase in numbers of swollen conidia is ascribed to a slow leakage of sucrose into the conidia.

The osmotic potential of the sucrose solution producing 50% swelling after 40 min was −89·5 bars, in comparison with the value of −121·6 bars found for the osmotic potential of conidia in Section III(*a*) above.

In order to test the effects of varying water potential on conidial germinability and viability, the following experiment was performed. Agar blocks (1 by 1 by 0·2 cm)

were soaked in sucrose solutions of various molalities for 2 hr. The blocks were then placed in fresh sucrose solutions for a further period of 2 hr before being placed on glass slides (Shepherd 1962), to ensure osmotic equilibration of agar and sucrose solution. Dry conidia were suspended in similar molalities of sucrose solution, as described in the experiment reported above, and placed on the agar blocks. After incubation at 20°C for 17 hr the degree of germination was assessed, 100 conidia being scored for germination from each of three replicate blocks at each sucrose concentration. The results (Table 1) indicate that the presence of external sucrose,

TABLE 1

EFFECT OF SUCROSE MOLALITY ON CONIDIAL GERMINATION

| Sucrose Concn. (molal) | Equivalent Water Potential (bars) | Germination after 17 hr at 20°C (%) | Sucrose Concn. (molal) | Equivalent Water Potential (bars) | Germination after 17 hr at 20°C (%) |
|---|---|---|---|---|---|
| 0 | 0 | 98·0 | 2·5 | −77·9 | 0 |
| 1 | −27·1 | 33·3 | 3 | −97·5 | 0 |
| 2 | −59·6 | 13·8 | 4 | −140·8 | 0 |

at the concentrations tested, markedly reduces germination. The degree of swelling of conidia after 17 hr was similar to that observed after 40 min incubation, indicating that equilibration was reached quite rapidly at the start of the experiment.

At the termination of the experiment, conidia were removed from the agar blocks and washed five times with water by centrifugation. When the viability of these washed conidia was tested by incubation at 15°C for 6 hr on fresh agar blocks (Shepherd 1962), virtually all conidia germinated, indicating that the lack of germination in the higher sucrose molalities was not due to a loss of viability of the conidia, but was due to the reduced water potential of the suspending media.

### (c) *Effects of Storage Temperatures and Humidities on Conidial Viability*

In order to determine the effects of storage conditions on viability, dry spores were dusted lightly on to 2 by 2 cm squares of Cellophane, which were placed in glass dishes in desiccators containing appropriate solutions for humidity control (see Section II). After storage at temperatures of 5, 15, and 25°C for various times, Cellophane and conidia were removed and the viability of the latter determined by adding a drop of water to the spores and incubating these at 15°C for 17 hr on the Cellophane on agar blocks. In a second experiment, conidia were allowed to commence germination (approximately 30% showing the production of germ tubes) on Cellophane on agar blocks, after which Cellophane and conidia were removed and dried overnight *in vacuo* over concentrated $H_2SO_4$. After drying, conidia and Cellophane were stored over humidity control solutions, as in the first part of the experiment. After various intervals of storage, the viability of these conidia was tested as above. Both experi-

ments gave virtually identical results, those from the first experiment being shown in Table 2, where it may be seen that both high temperature and high humidity have an adverse effect on conidial viability.

TABLE 2

EFFECT ON CONIDIAL GERMINATION OF STORAGE AT VARIOUS TEMPERATURES AND RELATIVE HUMIDITIES

Values indicate percentage germination. Least significant difference at 1% level = 8·2%

| Days of Storage | Relative Humidity (%) | | | Relative Humidity (%) | | | Relative Humidity (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 50 (−889·9)* | 80 (−286·4) | 0 | 50 (−921·9) | 80 (−297·0) | 0 | 50 (−953·9) | 80 (−307·3) |
| | Storage temp. 5°C | | | Storage temp. 15°C | | | Storage temp. 25°C | | |
| 0 | 97·6 | 98·0 | 100·0 | 100·0 | 98·4 | 99·0 | 98·2 | 99·6 | 98·8 |
| 5 | 74·7 | 74·5 | 69·5 | 71·5 | 65·4 | 63·0 | 55·7 | 54·9 | 53·3 |
| 10 | 46·2 | 32·4 | 32·0 | 44·4 | 28·7 | 28·3 | 19·7 | 19·2 | 14·4 |
| 17 | 34·3 | 22·8 | 22·3 | 28·9 | 20·8 | 20·3 | 14·9 | 12·6 | 10·4 |
| 30 | 25·9 | 10·1 | 11·2 | 19·0 | 10·4 | 10·4 | 7·3 | 2·7 | 1·0 |
| 37 | 22·2 | 8·2 | 6·6 | 12·6 | 3·6 | 5·8 | 1·5 | 0 | 0 |
| 45 | 16·8 | 1·0 | 1·6 | 8·6 | 1·6 | 0 | 0 | 0 | 0 |
| 50 | 8·0 | 0 | 0 | 1·0 | 0 | 0 | 0 | 0 | 0 |
| 60 | 2·1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 70 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* Values in parenthesis indicate equivalent water potential (bars).

In a second series of experiments conidia, which had been stored for 48 hr over calcium chloride (nominal 0% R.H.) or at 43% R.H. (−1142·0 bars) were placed in stoppered tubes. A suspension of $5\times10^4$ conidia/ml in water was placed in a third series of tubes. Tubes from each of the three series were placed in water-baths at

TABLE 3

INTERACTION BETWEEN TEMPERATURE AND WATER POTENTIAL AND THEIR EFFECTS ON THE VIABILITY OF *P. TABACINA* CONIDIA

| Temperature of Treatment (°C) | Percentage Germination of Conidia that had Received Temperature Treatments at: | | |
|---|---|---|---|
| | 0% R.H. | 43% R.H.* | 100% R.H. |
| 20 | 98·6 | 99·0 | 98·6 |
| 30 | 59·2 | 63·2 | 38·6 |
| 40 | 40·5 | 37·1 | 15·0 |
| 50 | 25·7 | 19·9 | 0 |
| 60 | 10·6 | 3·3 | 0 |

* The equivalent water potentials range from −113·7 bars at 20°C to −129·7 bars at 60°C.

20, 30, 40, 50, or 60°C. Following incubation for 10 min the tubes were removed, cooled to ambient temperature, and water added to those containing dry spores to give a suspension density of approximately $5\times10^4$ conidia/ml. Drops of the various suspensions were incubated on agar blocks at 15°C for 17 hr when the degree of germination was assessed by examining three lots of 100 spores from each treatment.

The results, shown in Table 3, indicate that conidia in aqueous suspension are more readily killed by high temperatures than conidia at lower water potentials.

*(d) Effects of Ambient Humidity on the Sensitivity of Conidia to Ultraviolet Irradiation*

The method used for determining the effects of ultraviolet irradiation on conidial viability has been described previously by Shepherd (1962). Conidia were suspended in water, allowed to equilibrate with the ambient humidity of the laboratory, or dried over anhydrous calcium chloride, prior to irradiation. It is evident from the results shown in Figure 4 that conidia containing reduced amounts of water are considerably more resistant to ultraviolet irradiation than conidia in water suspension.

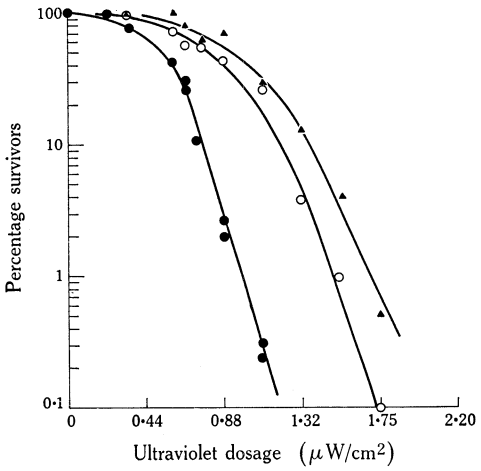


Fig. 4.—Effect of ultraviolet irradiation on viability of conidia.
● Conidia immersed in water.
○ Conidia held at 54% R.H.
▲ Dry conidia (0% R.H.).

*(e) Effects of Ambient Humidity on Rates of Fall of Conidia in Air*

To determine the effects of ambient humidity on rates of fall of conidia in still air, an apparatus was constructed of Perspex similar to that described by Ukkleberg (1933), except that the column was 2 m long and was insulated to ensure uniform temperature, as recommended by Weinhold (1955). The experiments were conducted in a constant-temperature room at 20°C, a 24-hr period being allowed for temperature equilibration between experiments. Humidities were measured by means of an aspirating psychrometer. Deposited spores were collected at 10-sec intervals, the method of Weinhold being used to assess mean rates of spore fall. Because of the length of time required for equilibration between experiments and because of the difficulties experienced in maintaining an adequate control of humidity, it was found expedient to use the two levels of humidity [34% (−1459·9 bars) and 79% (−318·9 bars)] that were found to remain relatively constant for long periods. [34±8% R.H. was the steady ambient level of the empty constant-temperature room at the time the experiments were performed, while the higher level of 79±12% R.H. was attained after the room had been filled with growing tobacco plants.] Ten observations were

made at each humidity level, the relation between rate of fall and ambient humidity being shown in Table 4.

TABLE 4

RELATION BETWEEN RATE OF FALL OF CONIDIA IN STILL AIR AND AMBIENT HUMIDITY

| Relative Humidity (%) | Equivalent Water Potential (bars) | Mean Rate of Fall* (cm/sec) |
|---|---|---|
| 34±8 | −1459·9± | 0·742±0·087 |
| 79±12 | −318·9± | 0·953±0·102 |

* Mean of 10 separate determinations.

## IV. DISCUSSION

While many studies have been made on the distribution of fungus spores as influenced by wind movement [see Gregory (1961) for review], very little work has been directed toward the study of factors influencing viability during dissemination. The delineation of these factors must be an integral part of any epidemiological study, for it is only the viable spore that has a potential for causing disease.

Sucrose at low concentrations enhances germination and growth (Shepherd 1962), but at higher concentrations germination is suppressed (Table 1). However, when the sucrose is removed, germination will continue [Section III(*b*)]. Thus, sucrose is not toxic to conidia and its action in suppressing germination may be ascribed to its effect of lowering the water potential of the conidia, which possibly leads to reversible changes in the macromolecules of the cell (Webb 1961; Falk, Hartman, and Lord 1963).

The epidemiological significance of the results is that, while germination may be arrested due to the low water potential of the site of a conidium on the plant, the conidium will remain viable for a considerable period of time. Were this water potential to be raised, by rain or by spray irrigation, to a level allowing germination, it is possible that establishment of the pathogen could occur at a time subsequent to the initial contact. The unsatisfactory nature of disease-forecasting systems based on the determination of period of conidial liberation in the field (Paddick, personal communication) may well be explained by the operation of such a mechanism.

Sussman and Halvorson (1966) provide data showing a direct relationship between longevity and water content of fungal spores, but instances are known where dehydration rapidly leads to death (Buller 1933; Glaz 1955). In the present study, the results shown in Table 2 indicate a strong interaction between the effects of storage humidity and temperature. Our results are not entirely in agreement with those of Hill (1962), who found that optimal conditions for longevity of *P. tabacina* conidia stored on tobacco leaf disks were at 5°C and humidities in the range of 30–40% (−1629·1 to −1239·9 bars) and that survival was shorter at zero humidity over the entire temperature range tested, than at humidites in the range 70–80% (−482·5 to −301·9 bars). The results of Darby and Mandels (1955) suggest that these

differences may be due to the differing chemical environments pertaining to the two experiments as medium constitution was shown by the above authors to have a striking effect on the longevity of conidia of a number of fungi.

The present results support those of Hill (1962), which contradict earlier work by Angell and Hill (1932) and Wolf *et al.* (1934). The statement by Rayner and Hopkins (1962) that conidia are short-lived and do not survive for more than 9 days under climatic conditions of 16–18°C and 60–80% R.H. (−691·2 to −301·9 bars) clearly does not apply to the strain of *P. tabacina* commonly found on tobacco in Australia.

Good and Zathurecsky (1967) found that the spores of several fungi could be germinated and then dried and still be capable of subsequent growth. This finding has been extended to include a similar behaviour of conidia of *P. tabacina* [Section III(*d*)]. Together with the ultraviolet resistance data, this finding indicates a considerably increased potential for disease production in the field over and above that ascribed previously to this organism by Rayner and Hopkins (1962).

According to Webb (1961) the response of microorganisms to radiation may be markedly affected by humidity. The considerable increase in resistance to ultraviolet inactivation following dehydration shown in the present study (Fig. 4) clearly has important epidemiological implications. The previous results of Angell and Hill (1931) and Shepherd (1962) indicate that conidia of *P. tabacina* would be rapidly killed by exposure to sunlight, but the fact that, during air dispersal, conidia would be partially dehydrated was not taken into account in assessing the epidemiological significance of their results.

Similarly, the increased resistance of conidia under conditions of low water potential to killing by high temperature may well be of considerable epidemiological significance, particularly during the summer growing period of tobacco in Victoria. Thus, the resistance to killing by both sunlight and high temperature suggests that conidia that had not lodged on a host plant after primary dispersal might still be viable following further windborne or other methods of redistribution, which allowed their subsequent contact with a host plant. While dispersal is often considered theoretically as a direct flight from source to host, the possibility of a "leap-frogging" mode of distribution cannot be dismissed.

In his original studies, Buller (1909) showed that the velocity of fall of mushroom basidiospores diminished as the spores fell after leaving the gills and suggested that the change in velocity was due to a decrease in the size of the spores because of loss of water by desiccation. Weinhold (1955) showed that the rate of fall of *Puccinia* uredospores increased with an increase in relative humidity. McCubbin (1918) and Ukkleberg (1933) have determined the theoretical distances for travel of spores in air and have shown that these distances would increase as the rate of fall of the spores decreased. The results shown in Table 4 indicate that increasing humidity leads to an increased rate of sedimentation of *P. tabacina* spores in still air. The results shown in Figure 2 indicate that conidial weight increases with increasing humidity. Thus, it might be expected that in air of low humidity conidia will be dispersed for greater distances than in air of high humidity, as it may be calculated from Stoke's law that a halving of the conidial mass will approximately halve its

terminal velocity in still air. Although the results of Rempe (1937) on pollen distribution at different altitudes suggest that the effect of such changes would be masked in the presence of air turbulence, as far as the authors are aware such mass changes in response to changing humidity have not been taken into account in the various theoretical treatments of spore dispersal in fungi.

Gregory (1961) has demonstrated that the efficiency of deposition of spores on surfaces is affected by the size of the spores, efficiency increasing with increasing size of spores. At first sight it might be expected that the lighter conidia found in air of low humidity would have less chance of deposition on plant surfaces than those found in air of high humidity. However, the observation made during this study of the extremely rapid response of conidia to humidity changes suggests that, during their passage within a crop, there might be an increase in mass with a concomitant increase in deposition efficiency. In the absence of further evidence, the effects of conidial mass changes on deposition cannot be elucidated.

## V. References


Angell, H. R., and Hill., A. V. (1931).—Blue mould of tobacco; longevity of conidia. *J. Coun. scient. ind. Res. Aust.* **4**, 181–4.

Angell, H. R., and Hill, A. V. (1932).—Downy mildew (blue mould) of tobacco in Australia. Bull. Coun. scient. ind. Res. Melb. No. 65. pp. 1–30.

Bromfield, K. R., and Schmitt, C. G. (1967).—Cryogenic storage of conidia of *Peronospora tabacina*. *Phytopathology* **57**, 1133.

Buller, A. M. R. (1909).—Rate of fall of fungus spores in air. *Nature, Lond.* **80**, 186–7.

Buller, A. M. R. (1922).—"Researches on Fungi." Vol. 2. (Longmans Green and Co.: London.)

Buller, A. M. R. (1933).—"Researches on Fungi." Vol. 5. Reprinted 1958. (Hafner: New York.)

Crozier, W. (1934).—Studies on the biology of *Phytophthora infestans* (Mont.) de Bary. Mem. Cornell Univ. agric. Exp. Stn No. 155. pp. 1–40.

Cruickshank, I. A. M. (1961).—Germination of *Peronospora tabacina*: effect of temperature. *Aust. J. biol. Sci.* **14**, 58–65.

Darby, R. T., and Mandels, G. R. (1955).—Effects of sporulation medium and age on fungus spore physiology. *Pl. Physiol., Lancaster* **30**, 360–6.

Falck, R. (1927).—Über die Grössen, Fallgeschwindigkeiten und Schwebewerte der Pilzsporen und ihre Gruppierung mit Bezug auf die zu ihrer Verbreitung nötigen Temperaturströmung-Geschwindigkeiten. *Ber. dt. bot. Ges.* **45**, 262–81.

Falk, M., Hartman, K. A., and Lord, R. C. (1963).—Hydration of desoxyribonucleic acid. 3. A spectroscopic study of the effect of hydration on the structure of desoxyribonucleic acid. *J. Am. chem. Soc.* **85**, 391–4.

Glaz, E. T. (1955).—Researches about the viability and preservation of the conidia of *Claviceps purpurea* (Fr.) Tul. grown in submerged culture. *Acta microbiol. hung.* **2**, 315–25.

Good, H. M., and Zathurecsky, P. G. M. (1967).—Effects of drying on the viability of germinated spores of *Botrytis cinerea*, *Cercospora muscae* and *Monilinia fructicola*. *Phytopathology* **57**, 619–22.

Gregory, P. H. (1961).—"The Microbiology of the Atmosphere." (Interscience Publishers Inc.: New York.)

Grindle, M., and Good, H. M. (1961).—Effects of drying on the viability of germinated and germinating conidia of *Monilinia fructicola* (Wint.) Honey. *Trans. Br. mycol. Soc.* **44**, 549–58.

Hill, A. V. (1962).—Longevity of conidia of *Peronospora tabacina* Adam. *Nature, Lond.* **195**, 827–8.

Hill, A. V. (1966).—Effect of inoculum spore load, length of infection period, and leaf washing on occurrence of *Peronospora tabacina* Adam. (blue mould) of tobacco. *Aust J. agric. Res.* **17**, 133–46.

KRAMER, P. J., KNIPLING, E. B., and MILLER, L. N. (1966).—Terminology of cell–water relations. *Science, N.Y.* **153**, 889–90.

MCCUBBIN, W. A. (1918).—Dispersal distances of urediospores of *Cronartium ribicola* indicated by their rate of fall in still air. *Phytopathology* **8**, 35–6.

MIDDLETON, W. E. K., and SPILHAUS, A. F. (1953).—"Meteorological Instruments." (Univ. Toronto Press.)

RAYNER, R. W., and HOPKINS, J. C. F. (1962).—Blue mould of tobacco: a review of current information. Commonw. Mycol. Inst. Misc. Publ. No. 16.

REMPE, H. (1937).—Untersuchungen über die Verbreitung des Blütenstaubes durch die Luftströmungen. *Planta* **27**, 93–147.

SHEPHERD, C. J. (1962).—Germination of conidia of *Peronospora tabacina* Adam. I. Germination *in vitro*. *Aust. J. biol. Sci.* **15**, 483–508.

SUSSMAN, A. S., and HALVORSON, H. O. (1966).—"Spores: Their Dormancy and Germination." (Harper and Row: New York.)

TERVET, I. A., RAWSON, A. J., CHERRY, E., and SAXON, R. B. (1951).—A method for the collection of microscopic particles. *Phytopathology* **41**, 282–5.

UKKLEBERG, K. A. (1933).—The rate of fall of spores in relation to the epidemiology of black stem rust. *Bull. Torrey bot. Club* **60**, 211–28.

VON STILLE, B. (1965).—Das Keimverhalten der Sporangien von *Phytophthora infestans* in Abhängigkeit von Temperatur- und Hydraturbedingungen. *Pflanzenkr. u. Pflannensch.* **4**, 193–200.

WEBB, S. J. (1961).—Factors affecting the viability of airborne bacteria. 5. The effect of desiccation on some metabolic systems of *Escherichia coli*. *Can. J. Microbiol.* **7**, 621–32.

WEINHOLD, A. R. (1955).—The rate of fall of urediospores of *Puccinia graminis tritici* Eriks. and Hen. as affected by humidity and temperature. Tech. Rep. Office of Naval Research. ONT Contract No. N90r.82400.

WOLF, F. A., DIXON, L. F., MCLEAN, R., and DARKIS, F. R. (1934).—Downy mildew of tobacco. *Phytopathology* **24**, 337–63.

WOODBURY, W., MACKO, V., and STAHMANN, M. A. (1957).—A collector for fungal spores. *Phytopathology* **57**, 455.