MYCOSPHAERELLA PINODES

II. THE PHENOLOGY OF ASCOSPORE RELEASE

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Summary

Continuous sampling within the foliage zone of peas grown in field plots at the Waite Institute in two seasons has revealed details of the fluctuation with time and with moisture supply in output of ascospores of *M. pinodes* (Berk. & Blox.) Vestergr. Dew moisture is shown to be effective in causing release of ascospores, but the largest airborne concentrations occur early in periods of rainfall.

Analysis of hourly ascospore counts on rainless days suggested a regular diurnal rhythm of ascospore release which was confirmed by sampling from a discrete source under controlled laboratory conditions. Treatments which minimized the diurnal changes in light intensity also reduced the magnitude of the diurnal changes in ascospore output.

The horizontal distance of projection of the ascospores in still air, the potential productive life of a perithecial substrate, and the period of high moisture required for infection of the host at four temperatures were determined in laboratory experiments. These properties of the organism are discussed in relation to its success as a foliage pathogen.

I. INTRODUCTION

In a previous paper, Carter and Moller (1961) described the broad pattern of variation in number of airborne ascospores of *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr. over an irrigated pea field, as determined by a series of spot samples at 0·5 m above ground level (a.g.l.) during two seasons. The results obtained suggested a need for a more detailed investigation of the fluctuations in ascospore output throughout the growth of a pea crop.

The present paper reports further studies of the relationships between ascospore release, moisture supply, and time, and gives the first account of an inherent diurnal rhythm of ascospore release in this species. It was not practicable to maintain the necessary instruments, which required daily attention, in a commercial crop at Paringa where the previous investigations were made: therefore plots of peas were grown for the purpose at the Waite Institute in 1961 and 1962.

II. FIELD STUDIES OF AIRBORNE ASCOSPORE CONCENTRATION WITHIN PEA CROPS

A plot of Greenfeast peas 10 m square was sown at the Waite Institute on March 13, 1961. On April 13, when the plants were about 15 cm high, infected pea straw was placed in a circle 1 m in diameter at the centre of the plot to act as a source of inoculum. Infection spread from this source, and by June 30 there were mature perithecia on senescent parts of the growing plants.

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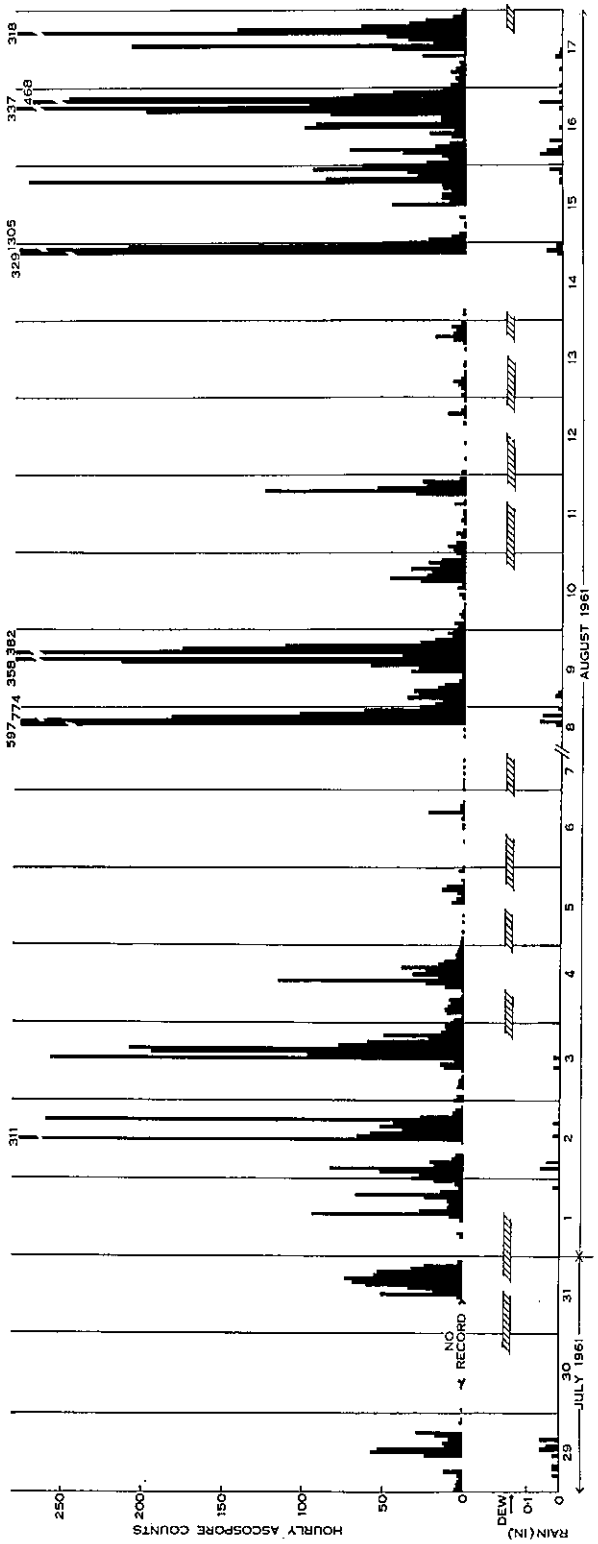

Fig. 1.—Hourly ascospore counts, rainfall, and dew periods in Waite Institute plot, 1961.

A Hirst spore trap (Hirst 1952) with orifice 15 cm a.g.l. was operated near the centre of the plot from July 4 to August 25, and a surface wetness recorder (Hirst 1957), with its recording surface at trap orifice height, was also operated within the plot from July 20 to August 25. Hourly records of rainfall were taken from a Friez continuous rainfall recorder at the Waite Institute meteorological station, approximately $\frac{1}{4}$ mile W. of the plot. It was thus possible to sample ascospores within the foliage zone of the crop and close to their source, giving a more precise measure of the fluctuations in airborne ascospore concentration resulting from environmental changes.

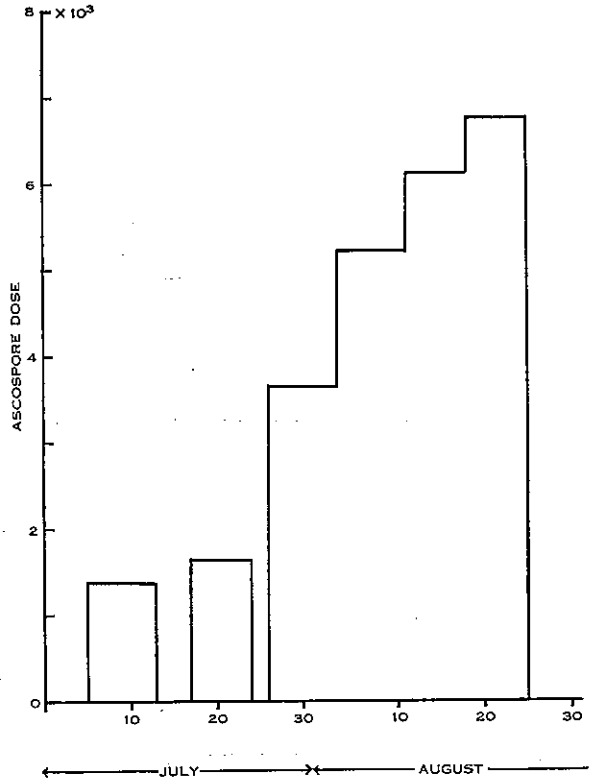

Fig. 2.—Ascospore dose in successive 7–8-day periods in Waite Institute plot, 1961.

The trap slides were mounted according to the method of Hirst (1953), a small quantity of cotton blue stain being added to the mountant to facilitate counting the ascospores. Counts for each hour were made in traverses 235 μ wide* across the slides at 2-mm intervals; those for two 9-day intervals are graphed in Figure 1, together with hourly rainfall data and dew periods indicated by the surface wetness recorder. Actual ascospore counts are plotted on the ordinate to eliminate additional conversions because the interest is in relative rather than actual concentrations. However, corresponding volumetric estimates may be obtained by multiplying the ordinate values by 14·18 to convert them to spores/m^3.

* Traverse width is normally equal to the diameter of a high-power microscope field. Different microscopes were used at times and hence a variation in traverse width occurs.

It is significant that (apart from two 24-hr periods when the trap was stopped) ascospores were detected every day in the air amongst the pea plants. Of these 51 days, 20 were rainless, yet ascospore concentrations of up to 4300/m^3 were recorded. There was a period of dew in each of the rainless days, but the response to dew was unexpected in that the highest ascospore counts on these days occurred approximately 1½ hr before the beginning of the dew period; this effect is discussed in Section V.

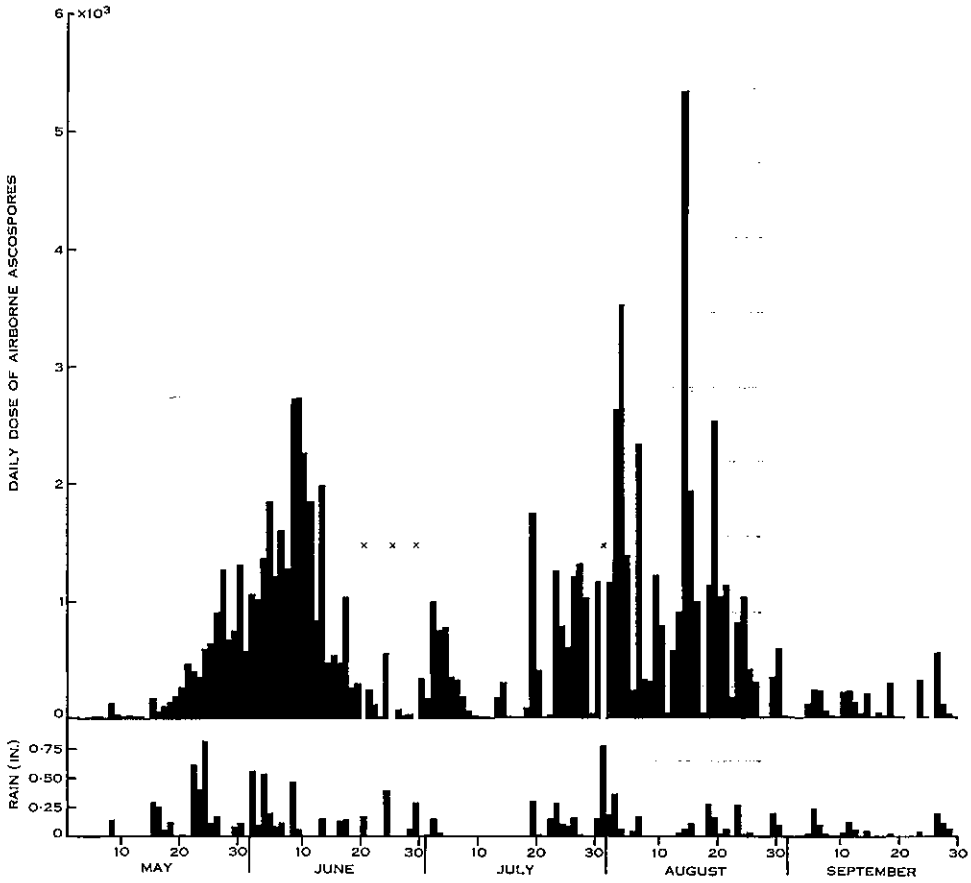

Fig. 3.—Daily rainfall and ascospore dose in Waite Institute plot, 1962. × denotes a missing record.

It is evident that at least a proportion of the perithecia within the plot received sufficient moisture for ascospore release on each of the 51 days, that ascospore release could occur at all times of the day and night (see Fig. 1), and that the largest counts occurred during the first few hours of rainfall which followed several rainless days, e.g. August 8 and August 14.

In order to determine whether there was a progressive increase in ascospore production in the plot during the 51-day period for which hourly spore counts are available, it is necessary to smooth out the violent fluctuations of airborne spore concentration associated with rains. This is shown in Figure 2 by plotting the ascospore "dose" (Hirst and Stedman 1961) which in this case is the summation of the hourly ascospore counts for successive 7- or 8-day periods. Trap failure is the

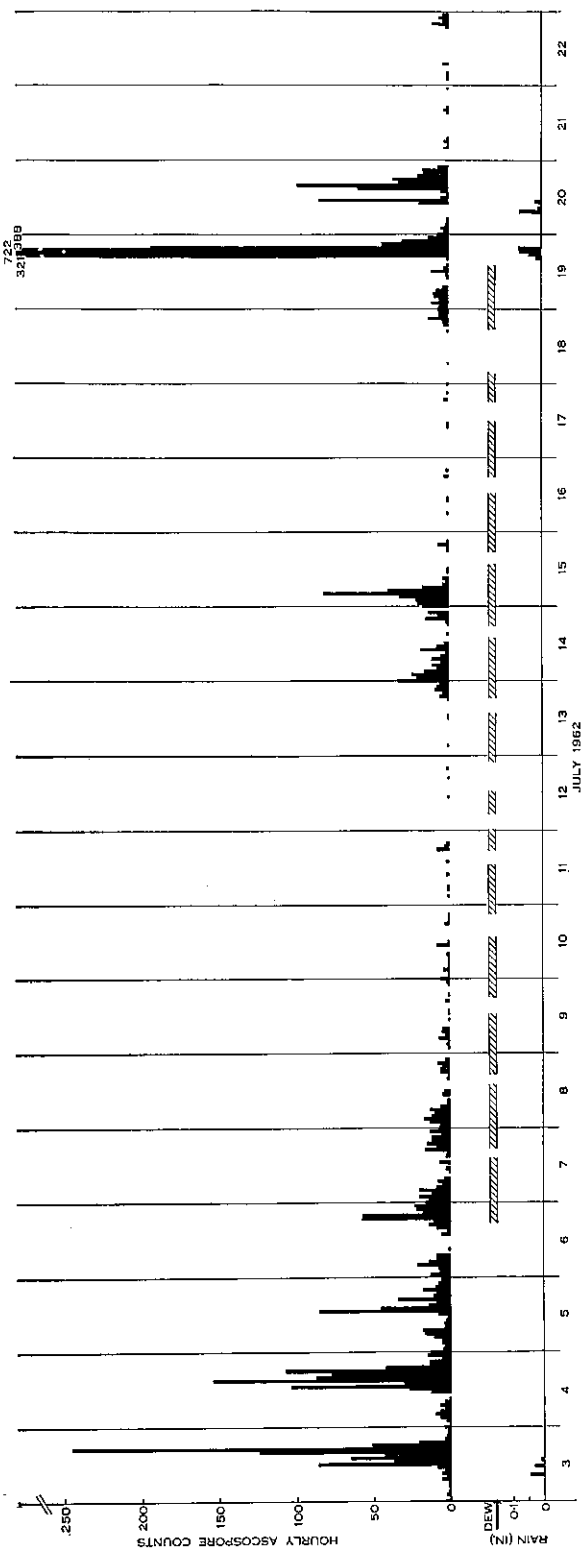

Fig. 4.—Hourly ascospore counts, rainfall, and dew periods in Waite Institute plot, 1962.

reason for some gaps in the July records, but there is a clear trend of increased ascospore production throughout the period studied.

The information gained in the 1961 experiment was representative only of the latter part of the life of the pea crop, so a similar experiment was performed in 1962 to study the pattern of ascospore output over a longer period. A plot of Greenfeast peas approximately 30 by 15 m was sown on March 14, and 2 weeks later, as the plants were emerging, infected pea straw from the previous year's crop was scattered throughout the plot. A Hirst spore trap with orifice 15 cm a.g.l. and a surface wetness recorder with recording surface at ground level were installed near the centre of the plot and operated from May 1 to September 30. A Casella natural siphon rainfall recorder, operating with a 24-hr chart, was placed at the edge of the plot. As in 1961, hourly ascospore counts were made from the trap slides, but the data were converted to daily estimates of ascospore dose by adding the 24-hourly counts for each day (1000 — 0900 hr); these are graphed, together with the daily rainfall data, in Figure 3.

After the maturation of perithecia on senescent leaves in the crop in mid-May, the daily ascospore dose increased rapidly to reach a peak on June 9–10. Contrary to expectation, however, and despite regular rains which fell during the remainder of June, the daily dose declined to a very low level by the end of the month. A second increase began early in July, but the dose declined rapidly during the 15-day rainless period of July 4–18. Thereafter the pattern is less clearly defined, but a further cycle of high ascospore output can be seen within the period July 20 – August 31, during which there were frequent rains.

The rainless period July 4–18, in which there was dewfall on all but two nights, has been selected for more detailed study, and the hourly ascospore counts and dew periods are graphed in Figure 4. The highest spore counts on July 4 and 5 occur in the afternoon, according to the usual pattern observed on rainless days in 1961. The next 7 days show a marked decline in ascospore numbers and a less well-defined pattern, and from July 13–18 ascospores were detected only within the periods of dew wetness.

III. Effect of Dew in Releasing Ascospores

The effect of dew in releasing ascospores has interested several workers with *Venturia inaequalis* (Moore 1958; Hirst and Stedman 1962). The latter authors, however, concluded that although "dew release" may be demonstrated experimentally, the number of airborne ascospores of *V. inaequalis* which can be detected close to apple leaves during periods of dew is always many times less than the number during and after brief intervals of rain.

In Section II it is shown that the release of *M. pinodes* ascospores may continue for many days in the absence of rainfall when frequent dews occur. It is of interest, therefore, to determine whether the moisture contributed by dew is sufficient *per se* to maintain the perithecia above the critical moisture content for ascospore release. The experiment now to be described was performed at the Rothamsted Experimental Station, England, during the autumn (September) of 1960, and was referred to in

the Rothamsted Annual Report for 1960 (Carter 1961). It is described here in its natural context in preference to its correct chronological sequence in relation to Section II.

A Hirst spore trap was modified by removing the horizontal rainshield and attaching a frame to the orifice cylinder which enabled a quantity (approx. 50 g) of pea straw to be carried level with and upwind of the orifice. The straw was arranged in the shape of a sector with apex towards the orifice. The trap was set in an open area over short grass, with the orifice 0·5 m a.g.l., and a Mk. III surface wetness recorder was placed nearby to record the duration of dew deposits at this level.

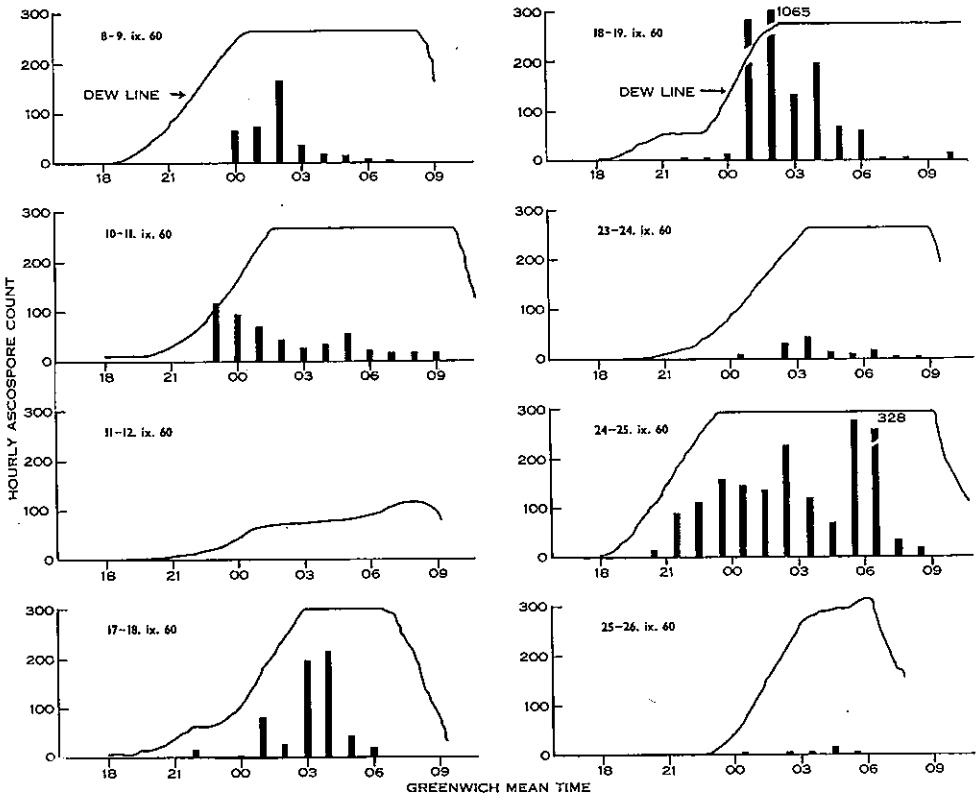

Fig. 5.—Hourly ascospore counts and dew periods, Rothamsted Experimental Station, England, 1960.

The pea straw was exposed only on nights when dew was expected, and it was removed to cover each morning between 0900 and 1000 hr, the same straw being used on each occasion. Hourly estimates of ascospore concentration were made by counting the ascospores in traverses 422 μ wide across the trace at intervals 2 mm apart. The counts for each of eight nights on which a dew was recorded are illustrated in Figure 5 as histograms superimposed on reproductions of the surface wetness recorder charts.

It may be seen from Figure 5 that ascospores were detected on each of the seven nights when a heavy (full-scale deflection) dew was recorded, while on the one night (Sept. 11–12) when a very light dew occurred, no ascospores were detected. Ascospores were usually detected within 3 hr of the beginning of dew formation (as recorded by the instrument) and they were never detected after the time when the dew line fell below the top of the scale.

The experiment served to demonstrate beyond doubt that dew moisture *per se* is sufficient to bring about the release of ascospores of *M. pinodes* from pea straw which is "air-dry" prior to dew formation each night, and also that the concentration of airborne ascospores thus released may reach a high level close to the source; the highest estimate recorded was 8500/m^3 on the night of September 18–19.* However, quantitative interpretation of the data should be made with caution for the following reasons: (1) During periods of dew formation, wind velocity and turbulence at this level are likely to be very low; hence deposition of many spores is likely to take place by sedimentation within a few centimetres from their point of release. (2) The moment of inertia of the Hirst spore trap may often be in excess of that which can be overcome by the rate of air movement prevalent during a dew period, and it is conceivable that the trap and its attached source could often remain at rest with the source "downwind" of the orifice for several hours at a time during dew periods. The lack of any regular pattern of variation in the estimated ascospore concentrations is attributed tentatively to this cause.

Although the surface wetness recorder is not designed to give a quantitative measure of dew deposits, nevertheless the normal method of testing its sensitivity is to place a 2 g weight on the recording surface (Hirst, personal communication): this should give a full-scale deflection of the pen on the chart. From the area of the polystyrene recording surface one may calculate the rainfall equivalent to a full-scale deflection as approximately 0·018 in., and that for a half-scale deflection as 0·009 in. *M. pinodes* ascospores were usually detected before the instrument reached half-scale in dew periods, and this makes an interesting comparison with the rainfall of 0·007 in. required to produce measurable ascospore release from air-dry pea straw in wind tunnel tests (Carter and Moller 1961).

IV. Period of High Moisture Required for Infection from M. pinodes Ascospores

Hare and Walker (1944) stated that at least 6 hr in a moisture chamber was required for infection of pea plants following the application of spores of *M. pinodes*. However, they did not state whether their inoculum was pycnidiospores or ascospores, nor did they specify the temperature within their moisture chamber.

An experiment was designed to determine more precisely the period of exposure to high moisture required for infection at four temperatures. Ascospores were released on to pea shoots standing in small tubes of water; the shoots were then sprayed with water from an atomizer and placed in closed containers in incubators at 5, 10, 15, and 20 °C. A pilot experiment gave the approximate period required to produce

* Volumetric concentration obtained by multiplying ordinate values in Figure 5 by 8.

lesions at each temperature; in further, more precise tests, shoots were removed at hourly intervals and dried quickly in front of a fan before inspection. Minimum periods of 46–47, 7–8, 5–6, and 4–5 hr were required at temperatures of 5, 10, 15, and 20 °C, respectively. When infection occurred, macroscopically visible lesions appeared on pea leaves within 24 hr after removal from the moisture chamber when the plants were kept at room temperature (15–20 °C).

V. Diurnal Periodicity of Ascospore Release

A diurnal periodicity of ascospore release is a common phenomenon in Pyrenomycetes and has been studied in detail by Ingold and his co-workers (Ingold 1933; Ingold and Cox 1955; Ingold and Dring 1957). In every species investigated, Ingold (1933) found clear evidence of diurnal periodicity of spore output, some species having a night maximum and others having a day maximum. Light appeared to be the master factor determining the periodicity, but the response to photoperiod varied from one species to another.

Ingold's experiments were all performed in the laboratory under controlled conditions; in addition to these there are the extensive data of Gregory (1952*a*) and Hirst (1953) obtained by operating high-efficiency suction traps in the field, from which it is seen that each component of the air spora has a characteristic diurnal periodicity in its airborne concentration.

The hourly counts of *M. pinodes* ascospores caught by a Hirst trap at 15 cm a.g.l. in 1961 (Section II) gave the first suggestion of a regular diurnal variation in number. The graphed data for rainless days (see Fig. 1: July 31, August 4, 5, 6, 10, 11, 12, 13) show a distinct late afternoon peak, and when the hourly data for 15 such days are plotted as geometric means according to the method of Hirst (1953) a marked peak occurs at 1730 hr (Fig. 6). As this peak did not coincide with any observable fluctuation in the supply of moisture to the perithecia (e.g. dew) nor come at a time of day when the maximum atmospheric turbulence would be expected to increase the sampling efficiency, a regular inherent diurnal rhythm was suspected, and the following laboratory experiments were designed to test this hypothesis.

A small wind tunnel was constructed from galvanized iron, with a clear "Perspex" top, having dimensions similar to those used in the quadruple ascospore-liberation tunnels described by Hirst and Stedman (1962), and air was drawn from this through a Hirst trap. The laboratory experiments were designed to study the hourly variations in ascospore release from perithecia in pea straw after the following variables had been minimized:

(1) *Temperature*—by operating the apparatus in a laboratory with temperature control accurate to $\pm$ 4 °C.

(2) *Moisture*—by placing the source material on a bed of continuously moist sand fed from a water-bath below.

(3) *Atmospheric turbulence*—by sampling air drawn continuously at a constant flow rate through the tunnel.

Three laboratory experiments were performed, viz.:

Experiment 1: In the natural daylight cycle, to compare results with 1961 and 1962 field data.

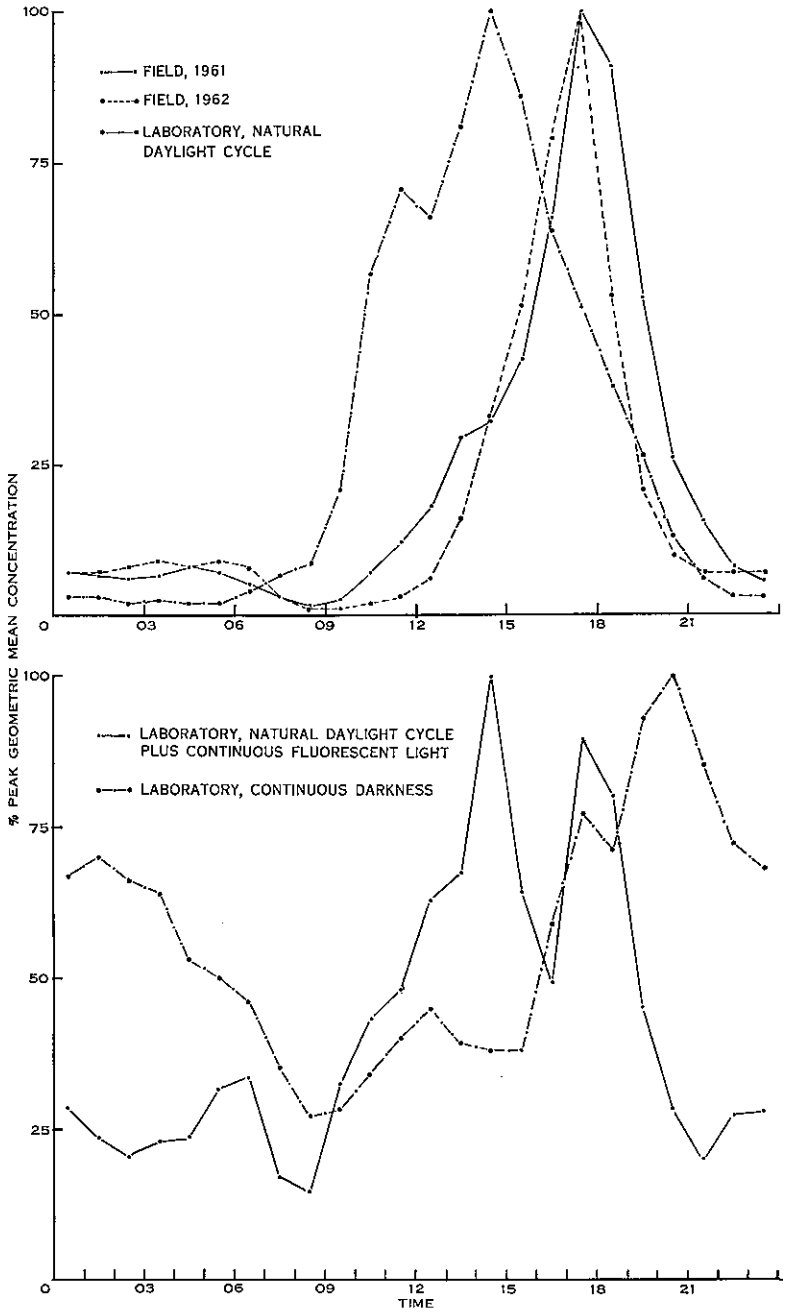

Fig. 6.—Diurnal periodicity curves for *M. pinodes* ascospores, drawn from field and laboratory data.

Experiment 2: In continuous fluorescent lighting superimposed on the natural daylight cycle. Two 40-W "daylight" fluorescent tubes above the "Perspex" top of the tunnel gave approximately 200 f.c. illumination at the level of the ascospore source.

Experiment 3: In continuous darkness, with black polythene covering the "Perspex" top of the tunnel, and with a light trap between tunnel and spore trap.

Ideally, experiment 2 should have been performed in the absence of natural daylight, but darkroom facilities adequate to accommodate the bulky apparatus for a 2–3-week period were not available.

In the upper portion of Figure 6 are shown the diurnal periodicity curves drawn from the two seasons' field data for rainless days together with that from laboratory experiment 1 (natural daylight). Curves from the remaining two laboratory experiments are shown in the lower portion of the same illustration. The curves are drawn from 15 days' data in all but laboratory experiment 3; for this experiment there were only 9 days' data because of difficulty in maintaining the sand bed uniformly moist owing to a heavy growth of mycelium on its surface. In each of the two laboratory experiments involving a modification of the natural daylight cycle, a 2–3-day conditioning period was allowed before spore counts began. Each curve is drawn from the geometric means of the sums of the hourly ascospore counts (Hirst 1953).

(i) *Field Data*—The curves for the two seasons' data bear a close resemblance, and both show a sharp peak at 1730 hr and a very low rate of ascospore release between 2200 and 1000 hr.

(ii) *Laboratory Experiment* 1 (*natural daylight cycle*)—The curve shows a similar trend to that obtained from the field data, but the peak ascospore release occurred at 1430 hr, 3 hr earlier than in the field. The experiment was conducted between December 13 and January 19 (about the summer solstice), whereas the field data are all drawn from the period May 27–August 24 (about the winter solstice); therefore the earlier and longer photoperiod in the summer is thought to account for the earlier peak discharge. The small subsidiary peak at 1130 hr is tentatively attributed to a short interruption to the dark period each night between midnight and sunrise when the laboratory lights were turned on for approximately 10 min by the cleaning staff.

(iii) *Laboratory Experiment* 2 (*continuous fluorescent light plus natural daylight*)—The periodicity curve is strongly bimodal, with peaks at 1430 and 1730 hr. The maximum output at 1430 hr is taken to be a response to the natural photoperiod, as the experiment was performed in the same season as experiment 1. It is not possible to give a logical explanation of the second peak 3 hr later, but it seems to be a result of the fluorescent light treatment, as does the increased ascospore output between 2200 and 1000 hr.

(iv) *Laboratory Experiment* 3 (*continuous darkness*)—Again there is an evening peak (2030 hr) in the periodicity curve, and in marked contrast with all other treatments is the maintenance of a high ascospore output throughout the night. At no time of day was the output less than 25% of the maximum. No logical explanation can be found for the occurrence of the peak at 2030 hr; the experiment was per-

formed in January and had there been an undetected leakage of light, one would expect a peak discharge earlier in the afternoon.

VI. Horizontal Distance of Projection of Ascospores in Still Air

Ingold (1956) and Ingold and Hadland (1959) have studied the distance to which the spores of certain Ascomycetes may be projected following rupture of the ascus, and in general the larger the spore, the greater is this distance. The active discharge mechanism in the Pyrenomycetes provides a very efficient means of launching their ascospores across the laminar boundary layer of air overlying the substrate within which they are formed (Gregory 1952*b*), and the greater the distance

Table 1

Number of M. pinodes ascospores counted in successive 1-mm intervals from perithecia after discharge in still air

Straw No.	0–1 mm	1–2 mm	2–3 mm	3–4 mm	4–5 mm	5–6 mm	6–7 mm
First test							
A	32	30	16	14	15	0	0
B	40	15	33	46	64	14	1
C	25	38	53	40	28	12	0
Total	97	83	102	100	107	26	1
Total (as %)	19	16	20	19	21	5	0
Second test							
A	36	40	20	13	21	4	
B	23	36	33	11	5	0	
C	48	91	99	51	20	5	
D	23	31	46	31	10	2	
E	37	52	54	37	8	0	
Total	167	250	252	143	64	11	
Total (as %)	19	28	28	16	7	1	
Average of two tests (%)	19	22	24	17·5	14	3	

of projection the more likely are the spores to be carried away from their source under calm atmospheric conditions, for within the laminar boundary layer the wind speed increases linearly with height (Gregory 1961).

The following technique was used to determine the horizontal distance to which *M. pinodes* ascospores may be projected in a still atmosphere. Short, straight pieces (approx. 1·5 cm in length) of pea straw bearing perithecia were inserted vertically into the surface of water agar which had previously been stored at 20 °C in 9-cm petri dishes for several hours. Each straw was banded with paraffin wax to preclude discharge from perithecia situated in the 2–3 mm of its length immediately above the

agar. Discharged spores were thus expected to follow normal trajectories resulting from their horizontal force of projection from the ascus and the downward force of gravity. After the straws had been inserted in the agar, the dishes were covered and left undisturbed for a period of 20 hr after which the straws were removed. The proportion of spores which fell in each successive 1-mm annulus was then estimated by counting along eight equally spaced radii, at a magnification of ×120. The experiment was performed twice and the results are presented in Table 1.

It is seen that approximately equal proportions of spores discharged from the vertical straws were deposited in each of the first five 1-mm annuli radiating from the edges of the straws, and that 95–97% of the spores were deposited within 5 mm of the perithecia from which they were discharged.

VII. Productive Life of a Perithecial Substrate

The author has seen few experimental data concerning the period of productivity of perithecia in the Pyrenomycetes, yet the biological consequences of either a long or a short productive life may be great. *Venturia inaequalis* may be cited as an example of a short productive life; Hirst and Stedman (1961) have shown that ascospores of this fungus can be detected in the air of infected orchards on fewer than 30 wet days following the maturation of perithecia each season. Provided that adequate prophylactic or therapeutic measures have been practised during the early part of the season, later sprays may be omitted without serious risk of fruit infection. On the other hand, Carter and Moller (unpublished data) have found that perithecia of *Eutypa armeniacae* on the same piece of apricot wood appear to be active for at least 9 months of the year. Whether the same perithecia remain active throughout this period or whether successive crops of perithecia mature and degenerate is not certain and would be difficult to determine. It seems clear, however, that perithecia of *Eutypa* do not remain active for more than one year and that new perithecia are formed in successive years in the same stromatic tissue (Carter and English, unpublished data).

Pea growing in the Renmark district of South Australia is based on a system of monoculture because of the peculiarities of market prices and the economics of irrigation installations (Carter and Moller 1961). In other districts, crop rotation is practised to a greater extent, but it is essential to know the length of time that the fruit bodies of *M. pinodes* may survive in order to plan a crop rotation to meet the needs of any district.

Hare and Walker (1944) found that *M. pinodes* perithecia remained viable for about 18 months when straw was kept in a location protected from the weather, but they did not state whether their samples were subjected to periodic ascospore discharge.

A sample of infected pea straw was collected from the field at Paringa on February 11, 1958, and three subsamples were tested for ascospore discharge on 33 occasions (at least once in each calendar month) between February 14, 1958, and August 10, 1959, using the method of Carter (1959). The samples were allowed to dry in the laboratory between tests. The sampling method was not suitable for making quantitative estimates of ascospore output; however, ascospores were

detected at each test throughout the 18-month period and there was an apparent overall decline in ascospore output with time.

The experiment was subsequently repeated, using a set of quadruple ascospore-liberation tunnels (Hirst and Stedman 1962). Four replicate samples of pea straw, each containing 45–50 pieces, 2·5–5 cm in length (approx. 4 g air-dry weight of straw per sample) were stapled to strips of fibreglass mesh. Beginning on May 26, 1961, and at 2- to 4-weekly intervals thereafter, the samples were immersed in water

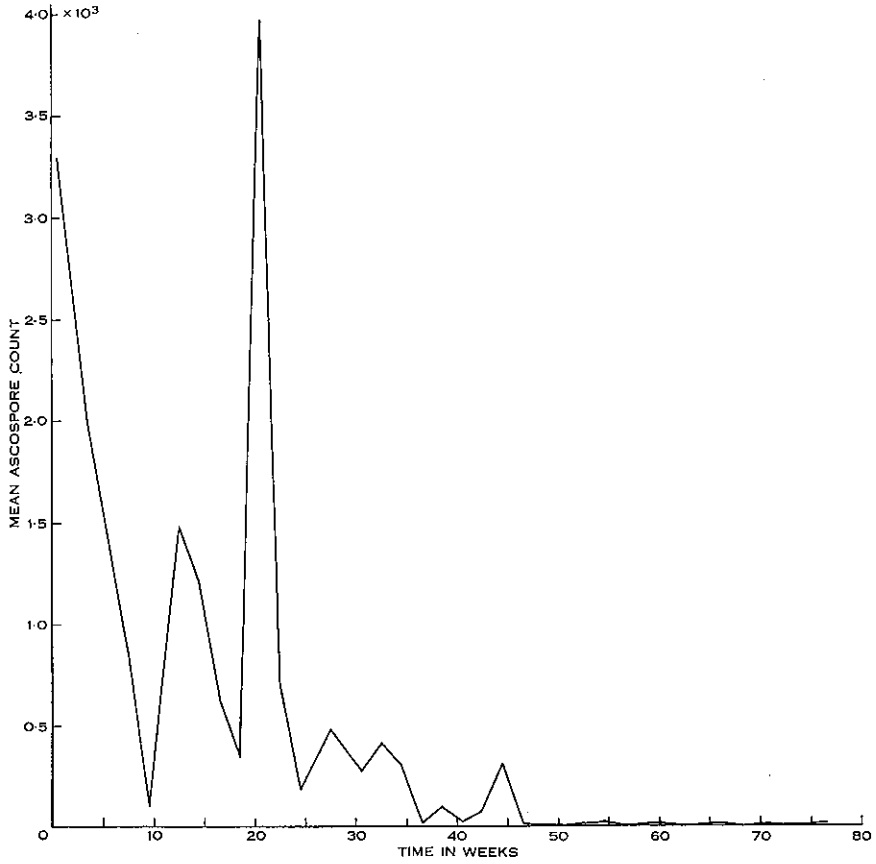

Fig. 7.—Ascospore output from pea straw samples as determined in quadruple ascospore-liberation tunnels.

for 5 min and then placed in the sampling apparatus for 2 hr. Between sampling dates, the samples were left to dry on the laboratory bench. The ascospore output from each sample was estimated by counting the spores in 15 traverses, 235 μ in width and 1 mm apart, across the deposit on the sampling slide. There are losses due to deposition along the length of the sampling tunnels (Hirst and Stedman 1962) so that estimates based on these counts will underestimate the actual ascospore output from the samples. Because the interest is in relative output rather than absolute output, the mean count for each sampling date is plotted in Figure 7. The

highest count scored (3973 at the ninth sampling) represents an output of more than 740,000 ascospores per sample. This is equivalent to 185,000 ascospores per gram of straw, or approximately 5000 per 1 cm length of straw.

The data illustrated in Figure 7 show a distinct rise and fall of ascospore output with time, suggesting maturation of successive crops of perithecia or of ascospores or of both, culminating in practical exhaustion of the perithecia and failure to regenerate after 50 weeks, when only occasional ascospores were detected. A similar rise and fall in spore output was noted in the 1962 field experiment (Section II, Fig. 3) in which there were two well-defined cycles of high output in a period of 5 months.

VIII. Discussion

Jones (1927) and Hare and Walker (1944) established the importance of pea crop residues in carrying over *M. pinodes* from one season to the next, and Carter and Moller (1961) have drawn attention to the difference between the oversummering function of the perithecia under conditions peculiar to the irrigated pea-growing districts of South Australia and their overwintering function in colder regions of the northern hemisphere where pea crops are sown in the spring. In Wisconsin, U.S.A., the fruit bodies of the fungus are not productive at the end of the winter season (Hare and Walker 1944) but new fruit bodies (perithecia and pycnidia) are produced in the spring. In South Australia, productive perithecia have been collected from the field in all seasons of the year.

Hare and Walker (1944) and Baumann (1953) seem to be the only workers who have been concerned with the relationship between moisture and release of *M. pinodes* ascospores: the former authors found that ascospores were never detected on slides exposed 3 ft a.g.l. in infected pea plots except after rains, and the latter stated that ascospores are released only in the presence of water drops. The seasonal variation in airborne ascospore concentration and its relationship to the variation in moisture conditions in the crop have not been reported previously: it is now clear that the expected seasonal pattern in South Australian irrigated pea fields is that of a decreasing supply of inoculum from the time the crop is sown until new perithecia mature (Carter and Moller 1961) followed by a rapid increase in the supply. There is evidence from the 1962 Waite Institute experiment that the supply may then rise and fall in a cyclical manner with a period of 6–8 weeks, suggesting a successive maturation and exhaustion of new generations of perithecia. The pattern of ascospore output with time revealed by the laboratory test of straw samples (Section VII) also supports this view.

The apparently long productive life of a sample of pea straw tested at regular intervals in the laboratory does not necessarily imply a similar term of productivity in the field, where more frequent exposure to moisture may hasten the process of exhaustion, and where the greater abundance of other organisms would cause more rapid decomposition of the straw. It is likely that the productive life would be related to the amount and distribution of the annual rainfall, and that in low-rainfall localities such as the Paringa district of South Australia, undisturbed pea stubble may well remain an active source of inoculum for more than one year.

On rainless days, the only sources of moisture available to the perithecia are from dew, guttation droplets from vegetation, and from the soil. Hirst (personal communication) has stated that guttation water rarely runs away from the leaf tip under English conditions; if this be true generally, then the only circumstance in which this source of moisture might be available would be that where hydathodes of weeds are in very close proximity to the perithecia. The conditions for dew formation are also conducive to copious exudation of guttation water, and it would be very difficult in practice to separate the influence of the latter. No attempt has been made to do so in the field experiments reported in Section II, but the effect of dew alone has been assessed in the separate experiment reported in Section III.

In addition to the overall seasonal pattern, it is now clear that ascospore release is a continuous process for many weeks during the winter season, and not a discontinuous process, associated only with falls of rain, as assumed previously. The data for the 1961 field experiment (Fig. 1) also suggest a regular daily rhythm of ascospore release with an afternoon peak; in Section V this effect has been analysed and confirmed by laboratory tests in a controlled environment. The pattern of ascospore release from perithecia kept at relatively uniform temperature and moisture levels varied with changes in the photoperiod, and treatments which minimized the diurnal changes in light intensity and quality tended to raise the low level of output characteristic of the latter half of the dark period in a natural daylight cycle. A more exhaustive series of controlled-environment studies would be needed to elucidate the interaction between photoperiod, light quality, and the maturation and release of ascospores. It is possible that *M. pinodes* may tend to repeat its inherent diurnal rhythm in a changed photoperiod for longer than has been allowed for in the experiments reported here, e.g. Ingold and Cox (1955) showed that the periodic discharge characteristic of *Daldinia concentrica* in a natural daylight cycle is maintained for 12 days after transfer to continuous darkness. Technical problems of maintaining a uniform moisture supply, and exhaustion of the perithecia, prevented longer tests with *M. pinodes*. However, the diurnal periodicity postulated on the basis of field spore trap counts was found to be a real effect and cannot therefore be attributed to variations in atmospheric turbulence in the field. This finding adds confidence to the procedure of sampling with standard equipment within the foliage zone of a crop to gain information about the phenology of spore dispersal of an airborne pathogen whose source is within the crop.

The efficacy of the ascospore discharge mechanism in *M. pinodes* in launching the spores for air dispersal is borne out by the characteristic rapid spread of the foliage blight which follows maturation of perithecia in a crop. If we postulate a value of 1 mm for the average depth of the laminar boundary layer (Gregory 1961) we may then regard the 80% of *M. pinodes* ascospores projected 1 mm or more from their parent perithecia as being eligible for dispersal in eddy currents. Of those spores which are projected less than 1 mm, a further fraction may still reach the eddy layers of the atmosphere by gravity if they are released from perithecia elevated above ground level. Yet another fraction may be deposited by sedimentation on parts of pea plants situated below active perithecia.

Periods of maximum output of ascospores have been shown to occur during or shortly after rain and irrigation, and daily in the late afternoon of rainless days when

the substrate is sufficiently moist. Atmospheric turbulence would be moderate or high at such times and therefore would favour dispersal. The occurrence of a daily cycle of maximum output prior to the dew period ensures that the majority of spores deposited on foliage are exposed to the maximum period of surface moisture during the night hours, thus enabling germination to proceed within a short time after deposition.

M. pinodes requires only a short period of high moisture for its ascospores to germinate and penetrate the host, e.g. 4–5 hr at 20 °C. The current practice of irrigating summer-sown pea crops with sprinkler moves at 4–6-hr intervals therefore may be expected to increase greatly the number of infection periods to which a crop is exposed prior to the winter season, thus ensuring an abundance of new perithecia in the crop by the beginning of winter. Thereafter, the short life cycle of the fungus (Hare and Walker 1944) and the more frequent occurrence of rains and dews during a phase of continuous ascospore dispersal are likely to be complementary factors contributing to its success as a pathogen.

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