PRODUCTION AND DISPERSAL OF ASCOSPORES IN *EUTYPA ARMENIACAE*

By W. J. Moller* and M. V. Carter†

[*Manuscript received June* 30, 1964]

Summary

Perithecia and ascospores of *E. armeniacae* may be produced on dead apricot wood for at least 5 years following maturation of the first stromata. Each winter, a stroma may produce new perithecia between the exhausted ones of the previous year. Results from a three-year quantitative study of the seasonal abundance of airborne ascospores show that, in South Australia, there is a winter period of low ascospore frequency which coincides closely with the dormant period of the apricot host. Abundant ascospore release follows rainfall exceeding 0·05 in. at other times of the year. The pattern of ascospore release, when related to natural rainfall in field tests and to moisture supply in laboratory tests, gives no evidence of diurnal periodicity.

I. Introduction

Carter (1957*a*) established that *Eutypa armeniacae* Hansf. & Carter, an ascomycete abundant on dead wood of *Prunus armeniaca*, is the perfect state of a species of *Cytosporina* long known to be a pathogen of apricot trees in many districts of southern Australia. Evidence was produced that airborne ascospores, released during wet weather, accounted for the random distribution of infected branches which occurs within orchards and for the dispersal of the pathogen between orchards and districts.

In subsequent papers, Carter (1957*b*, 1960) reported *Vitis vinifera*, *Tamarix* sp., *Prunus amygdalus*, and *Pyrus malus* (syn. *Malus sylvestris*) as additional hosts for the perithecial stage and described the mode of infection from ascospores. Recently, Moller (1964*a*) has reported species of *Ceanothus* as further hosts.

This paper reports additional information about the productive life of perithecial stromata, the results of a three-year quantitative study of the seasonal abundance of airborne ascospores, and the relationship between ascospore release and moisture supply.

II. Productive Life of Perithecial Substrata

Carter (1963) has drawn attention to the scarcity of data concerning the productive life of perithecia in Ascomycetes, and the difficulty of distinguishing between the productive life of a stroma or substratum and that of individual perithecia within such a stroma or substratum. Evidence is now available that perithecia and ascospores of *E. armeniacae* may be produced on dead apricot branches for at least 5 years following maturation of the first stromata.

* Horticultural Branch, Department of Agriculture, Adelaide; present address: Department of Plant Pathology, University of California, Davis, California.

† Department of Plant Pathology, Waite Agricultural Research Institute, University of Adelaide.

Carter (1960) reported the developmental history of stromata and perithecia on 15 infected branches collected in 1955 and exposed in the open to natural rainfall at the Waite Institute. When that report was published, it was believed that these branches had been inadvertently destroyed before exposure to the fifth winter; eight of them, however, were rediscovered in February 1963, when they had then been exposed to the rainfall of eight winters. In February and March of 1963, samples of stromata from these eight branches were tested for productivity in quadruple ascospore-liberating tunnels (Hirst and Stedman 1962) and some stromata from one branch still yielded ascospores. Unfortunately the positions of the origiual stromata had not been marked, so it is not clear how much additional stroma had been produced during the 5 years after the first fructifications matured.

English and Carter (unpublished data) examined stromata in early spring, 1962, and found new immature perithecia developing between empty ones which presumably had matured the previous year. More recently, histological examination and concurrent periodic tests for ascospore release by one of us (W. J. M.) in 1963 confirmed the presence of both empty and apparently mature perithecia in individual stromata in winter. Immersion of such stromata in water, however, did not result in the release of ascospores into the air, which is normal following a thorough wetting at other times of the year.

III. Phenology of Ascospore Release

Carter (1957*a*) showed, by periodic air sampling in wet weather and by exposing tree wounds to natural infection for intervals of 4 weeks throughout a year, that there may be a low frequency of airborne ascospores in winter. In view of the implication of this to current South Australian apricot-pruning practice (late winter) a detailed study of seasonal frequency of airborne ascospores was desirable.

In May 1961, a Hirst spore trap (Hirst 1952) was installed in a commercial orchard at Light Pass (mean annual rainfall 21 in.) situated in the Barossa Valley, one of South Australia's main apricot-growing districts, where *E. armeniacae* fructifications are abundant. This trap was operated continuously for 30 months. Later a second trap was installed at the centre of an artificially placed, circular ascospore "source", hereafter referred to as the "ring source" (see Plate 1) and was operated continuously for 6 months in the Barossa Valley, then moved to the Waite Institute (mean annual rainfall 25 in.) for a further period of 18 months. A third trap was operated for 5 months during the summer and autumn of 1962–63 in a commercial orchard at Berri (mean annual rainfall 10 in.) approximately 100 miles ENE. of any known large source of *Eutypa* inoculum. The two traps in commercial orchards sampled air 2 m above ground level whereas the trap within the ring source sampled air 0·25 m above ground level; each was operated at a flow rate of 10 litres/min.

After exposure in the traps, slides were mounted in Solvar–lactophenol (Hirst 1953) to which cotton blue had been added, and hourly estimates of ascospore concentration were obtained by scanning short traverses (across the slide) 2 mm apart. Hourly rainfall records were also obtained from daily charts used in a Casella natural-siphon rainfall recorder at the site of each trap.

At first, difficulty was experienced in consistently distinguishing *E. armeniacae* ascospores from a variety of similar spore groups trapped during wet weather. Of the many biological stains tested, none stained these hyaline spores, and slight spore drift

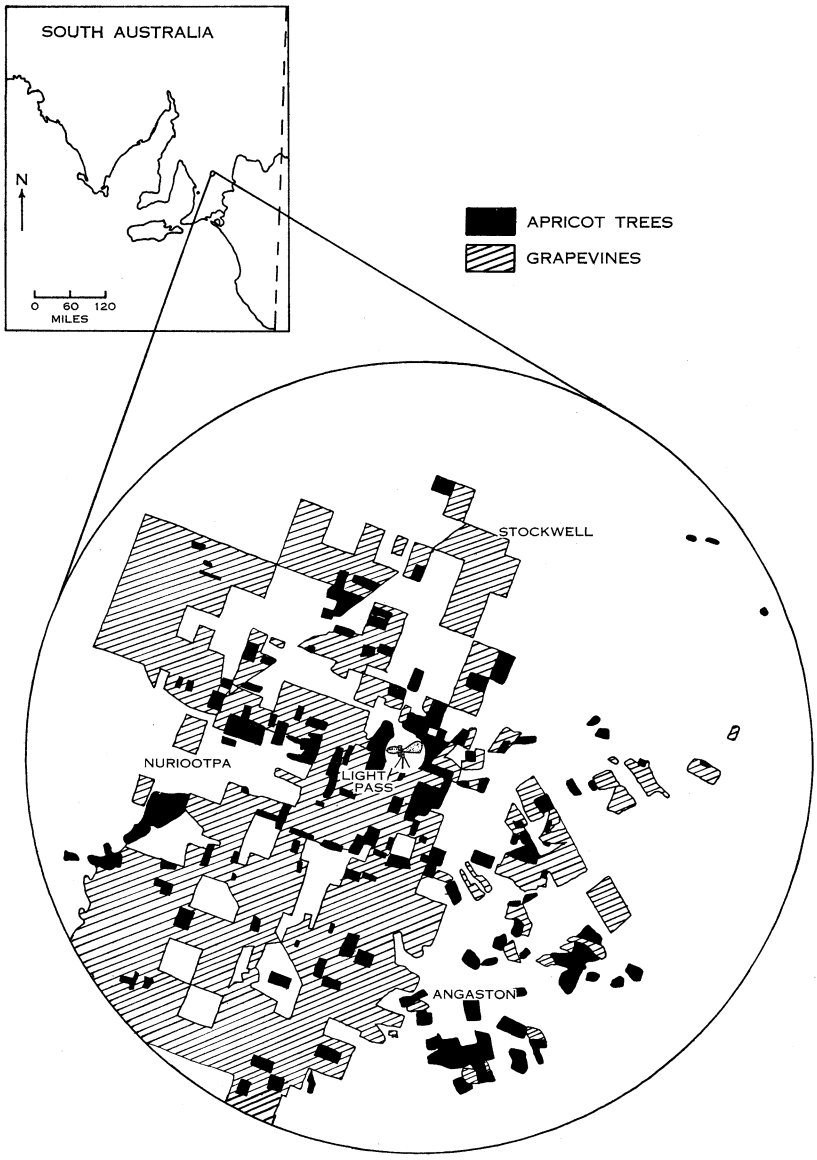


Fig. 1.—Positions of potential inoculum sources within a 4-mile radius of Light Pass spore trap in Barossa Valley, South Australia.

during mounting often made counting and identification [which depends on presence of the spores in octads (Carter 1957*a*), and on shape and size] extremely tedious. The presence of cotton blue in the mountant, however, was advantageous in two ways:

it stained certain other ascospores, also trapped as octads, whose size and shape closely resembled those of the non-staining *E. armeniacae*; it also provided a background against which the hyaline ascospores were easily seen. By using a mountant of high viscosity (saturated with Solvar), it was found that spore drift could be reduced to a minimum.

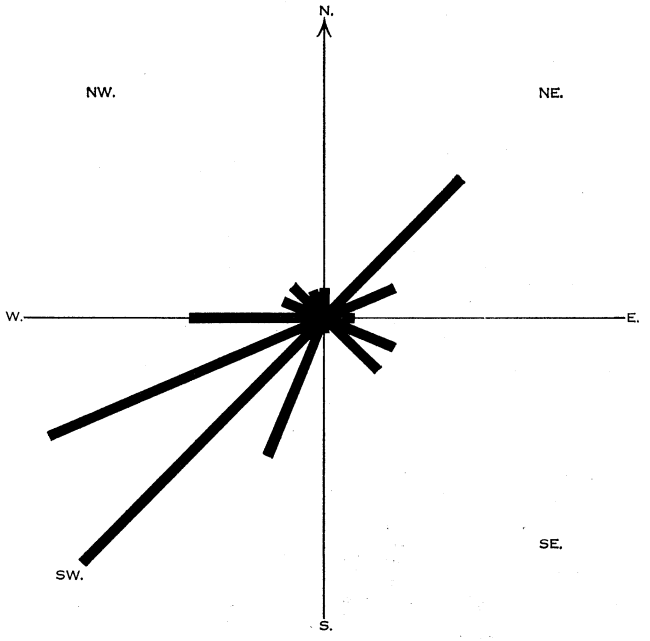


Fig. 2.—Wind-rose for Barossa Valley during rains exceeding 0·10 in. in the two-year period 1961–1963. Length of line at each compass point represents percentage frequency.

(*a*) *Frequency of Airborne Ascospores in Commercial Orchards*

(i) *At Light Pass*

The location of the Light Pass trap in relation to sources of inoculum is shown in Figure 1, while Figure 2 is a wind-rose showing the direction–frequency of winds in the district during rains which exceeded 0·10 in. The daily dose (Carter 1963) of ascospore octads, a summation of 24 (hourly) estimates of octads per cubic metre of air sampled each day, is plotted together with daily rainfall in Figure 3.

The first significant feature of the data is the low frequency, at all times, of airborne ascospores; in the 30-month period the daily dose exceeded 100 on four occasions only, and the maximum dose recorded was 375. A thorough search of the orchard in which the trap was situated failed to locate a source of inoculum, and the nearest known sources were approximately 400 m away. Therefore, the concentrations estimated at this distance from a source [cf. concentrations within ring source, Section III(*b*)], seem compatible with Gregory's (1945) theory of spore dispersion. In the absence of an estimate of the output of spores from the source, Q_o (Gregory, loc. cit.), however, it is not possible to analyse this situation further.

A second significant feature, apparent after the first year's sampling, was the absence of ascospores during the period mid-July to late September. Although this

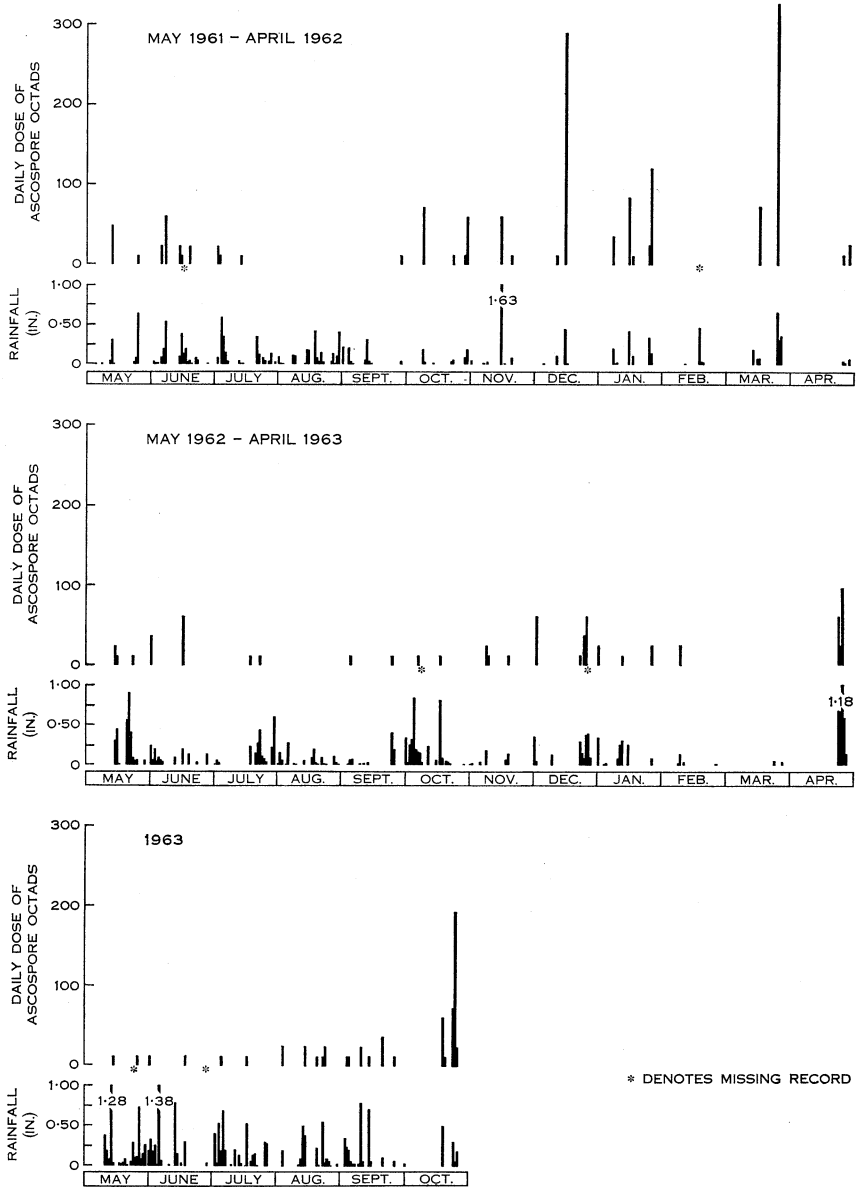


Fig. 3.—Daily dose of *E. armeniacae* ascospore octads and daily rainfall, Light Pass, 1961–63.

pattern was not exactly repeated in the next 2 years, there was nevertheless a suggestion that, despite frequent rains exceeding 0·05 in., a very low ascospore concentration is characteristic of the winter period.

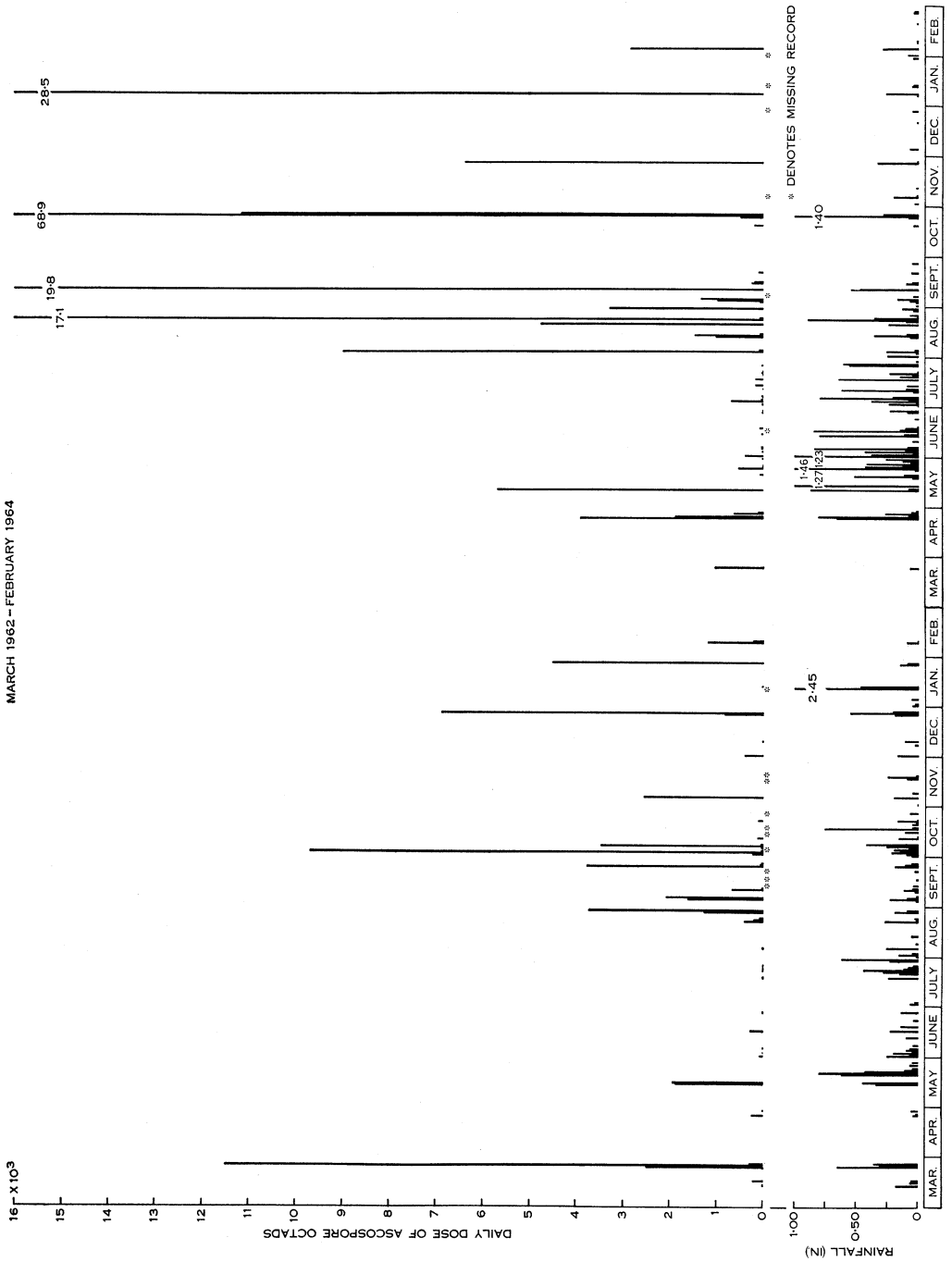


Fig. 4.—Daily dose of *E. armeniacae* ascospore octads and daily rainfall, ring source, 1962–64.

Thirdly, it was clear that the scanning procedure adopted had allowed only a narrow margin above the detection threshold (Hirst 1959) on the majority of days when ascospores were detected in the air. The mean daily dose for days on which ascospores were detected was 36 octads, this being only three times the value of the detection threshold. For this reason, the ring source was established in March 1962 to enable a more precise estimation to be made of the seasonal variations in ascospore output and of the relationship between rainfall and ascospore release [see Section III(*b*)].

(ii) *At Berri*

Carter (1957) noted the general absence of *E. armeniacae* perithecia from districts whose mean annual rainfall is below 13 in., and suggested that airborne inoculum from higher-rainfall districts up to 160 km away might account for the moderate amount of tree infection known to occur in the naturally arid, irrigated districts

TABLE 1

EXAMPLES OF DAILY ASCOSPORE DOSE RESULTING FROM COMPARABLE AMOUNTS OF RAIN IN WINTER AND SPRING OF 1963

Date	Days since Last Rain	Amount of Rain (in.)	Duration of Rainfall (hr)	Daily Ascospore Dose
June 6–7 (winter)	1	0·84	12	48
August 24–5 (spring)	1	0·89	24	17,000
July 27–8 (winter)	3	0·56	12	36
September 11–12 (spring)	3	0·54	21	20,000

bordering the River Murray. Berri, situated approximately 160 km ENE. of the Barossa Valley, was selected as a representative arid district, and continuous sampling was conducted between December 21, 1962, and June 1, 1963, on a property where apricots and grapevines are furrow-irrigated. Some *Eutypa* infection was apparent in apricot trees on this property. In periods of frontal rain, the normal wind pattern carries air from the Barossa district towards Berri.

During the period of sampling, a total of 8·77 in. of rain fell on 36 days; 18 of the falls exceeded 0·10 in. and were regarded as adequate for ascospore release [see Section III(*c*)]. The amount and time of rainfall at Light Pass was also noted, and slides from the Berri trap were scanned for appropriate intervals after both local rains and rains $>0\cdot10$ in. at Light Pass. Falls of rain at Berri coincided with those at Light Pass on 24 of the 36 days, but the amounts recorded at the two centres differed considerably. Rain at Berri frequently started falling 2–4 hr after the onset of rain at Light Pass, as the weather system moved eastwards.

No *E. armeniacae* ascospores were detected at any time by the Berri trap, confirming that the level of airborne inoculum in that district is below the detection

threshold of the trap, i.e. < 12 spores per cubic metre. This finding, although compatible with the hypothesis of long-distance dispersal of inoculum to the Berri district, does not substantiate it, for a few mature perithecial stromata present locally might give a similar result. During the period the Berri trap was operated, the mean daily dose of spores (wet days) at Light Pass was 28 octads.

(*b*) *Annual Periodicity of Ascospore Release*

The results reported above suggested a need for more detailed study of the seasonal pattern of ascospore output. Accordingly, in March 1962, a Hirst trap with orifice 0·25 m above ground level was placed at the centre of a 2 m diameter ring source, consisting of several pieces of dead apricot wood bearing abundant stromata of the pathogen (see Plate 1) collected in the Barossa Valley during the summer of

TABLE 2

RAINFALL AND DAILY ASCOSPORE DOSE RECORDS OF 1963 SHOWING ABRUPT CESSATION AND RESUMPTION OF OUTPUT

Date	Amount of Rain (in.)	Duration of Rainfall (hr)	Daily Ascospore Dose
May 11–12	0·87	9	5676
May 14–15	1·27	13	Nil
May 20–21	0·52	10	Nil
July 28–29	0·61	19	Nil
August 1–2	0·25	17	Nil
August 4–5	0·26	11	8976

1961–62. Sampling from this source during wet weather has been maintained to June 1964, and will be resumed in the spring of 1964 and autumn of 1965. Because of maintenance difficulties, the installation was moved from the original Barossa Valley site (mean annual rainfall 21 in.) to the Waite Institute (mean annual rainfall 25 in.) after the first 6 months. Slides were examined after exposure by the procedure described above, and the data for ascospore frequency and rainfall in the first 2 years are presented in Figure 4.

Sampling close to a discrete source of inoculum gave a much more precise measure of seasonal changes in ascospore output, and the low winter frequency suggested by the Light Pass data was confirmed. The magnitude of these seasonal changes can best be illustrated by tabulated examples from the 1963 data (see Table 1). In three successive years, a well-defined fall in ascospore frequency has occurred in May, and there has been an equally well-defined rise each August after 3 months of very low ascospore frequency. The abrupt end and beginning of the dispersal season may be illustrated by tabulated data from 1963 (see Table 2).

(c) *Pattern of Release Related to Rainfall*

Like those of many other Pyrenomycetes (Ingold 1960) the perithecia of *E. armeniacae* must be thoroughly wetted before their ascospores are released

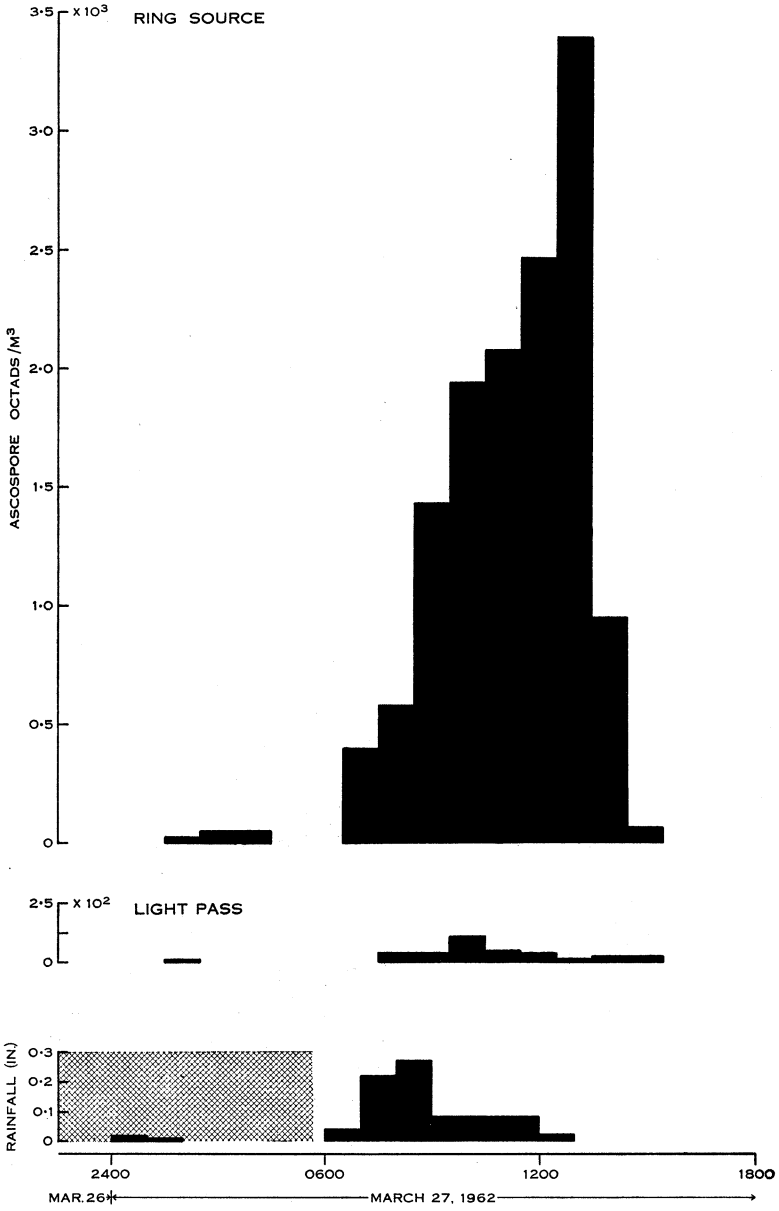


Fig. 5.—Hourly estimates of concentration of *E. armeniacae* ascospore octads and hourly rainfall, ring source, March 27, 1962. Cross-hatched area represents night period.

(Carter 1957*a*). Data from the ring source trap over a 2-year period showed that, in general, when rainfall was preceded by several dry days, a minimum fall of 0·05 in.

was necessary for ascospore release. The actual pattern of release following the onset of rain varied according to the weather conditions; a rainfall of 0·05 in. by late afternoon always produced a longer period of release than did a comparable amount of rain falling early in the day and followed by fine weather. Two examples from the ring

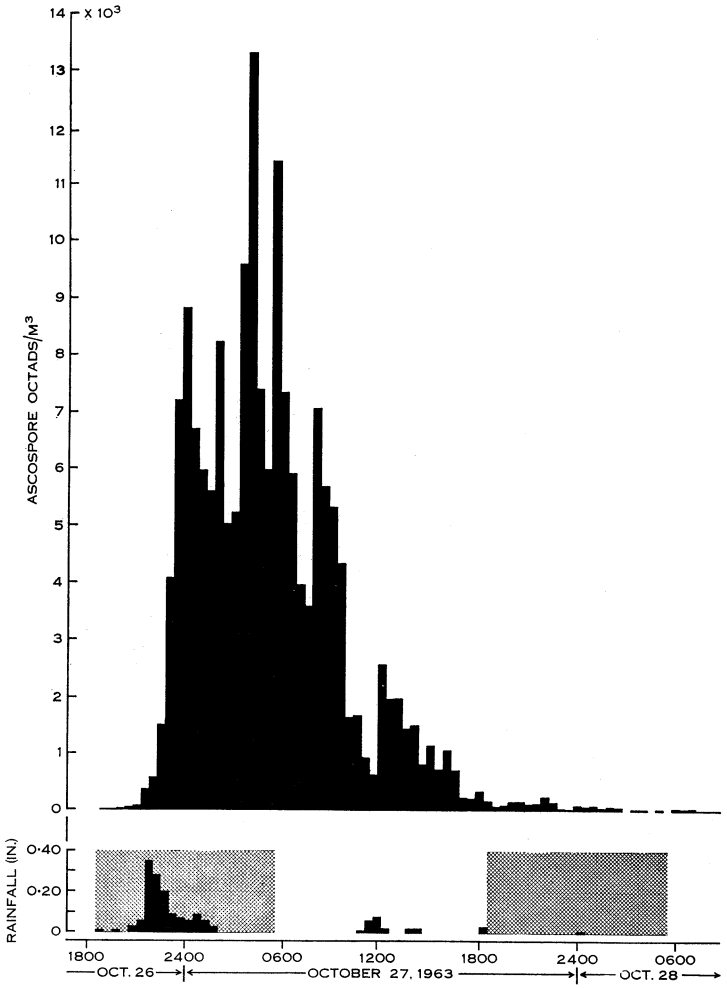


Fig. 6.—Half-hourly estimates of concentration of *E. armeniacae* ascospore octads and half-hourly rainfall, ring source, October 26–28, 1963. Cross-hatched area represents night period.

source are cited as illustrations of the release pattern; one of these is analysed on an hourly time-scale in Figure 5 and the other on a half-hourly time-scale in Figure 6. The cross-hatched area in each illustration represents the hours of darkness.

Figure 5 shows the data for March 27, 1962, from both the Light Pass trap and the ring source trap, the latter then being located in the Barossa Valley and subject to the same rainfall pattern as the former. On this occasion, no rain had fallen

in the district for 9 days and it could be assumed that all *Eutypa* stromata would have been dry when the rain began. Ascospore release was initiated by a light shower which fell 4 hr before the onset of steady rain and probably "conditioned" the perithecia for ready release [see also Section III(*d*)]. When heavy rain started at 0630 hr, ascospores were detected by both traps within an hour. Rain continued until 1230 hr and ascospores were detected until 1500 hr. This pattern is typical of daytime releases; it was noted many times that if rain ceased before or soon after midday, ascospore concentration would decline rapidly, presumably because of quick drying of the stromata. By contrast, if rain ceased at dusk or soon after, slower drying would allow ascospore release to continue for much longer. Ascospore concentration at the ring source on this occasion increased steadily for 7 hr, the maximum being reached half an hour after the rain ceased. The Light Pass trap detected ascospores for a similar period, but concentration was close to the detection threshold, and the rise and fall pattern was not apparent.

Figure 6 shows the analysis of ascospore concentration and rainfall on a half-hourly time-scale for October 26–28, 1963, when the greatest concentration for the 2-year period was detected at the ring source. Most of the rain fell during the first night (October 26–27) and a high ascospore concentration was attained by midnight. The maximum concentration was detected at 0400 hr on October 27, 2 hr after rain had ceased falling. Ascospore release continued for 38 hr and finally ceased on October 28, 8 hr after the last shower of rain.

These examples are typical of many periods (shown in less detail in Fig. 4) from which may be deduced several features of ascospore release. There is no suggestion of diurnal periodicity: during the dispersal season, sufficient rainfall at any time of the day or night gave rise to large ascospore concentrations within the ring source. Although ascospores were often detected within the first hour of rainfall (depending on the moisture content of the stromata prior to the rain) their concentration usually did not rise steeply until 2–3 hr later. There was often a clear relationship between amount of rain and ascospore concentration and, for any single wet period, a sudden change in the rainfall intensity frequently produced a corresponding change in concentration. The maximum concentration usually occurred 1–3 hr after rain had ceased falling. In extended periods of rain (see Fig. 4 — October 1–8, 1962; April 25–30, 1963; August 23–27, 1963) release may be almost continuous for up to 7 consecutive days, but a decline in output is usually noticed after the second consecutive wet day.

(*d*) *Pattern of Release in Laboratory Tests*

In order to study the moisture factor in greater detail, a number of pieces of productive stroma, each 5–10 cm^2 in area and attached to wood 0·5–1·5 cm thick, were cut from dead branches in the spring of 1962, and subjected to various laboratory tests.

In the first series of tests (Table 3, Nos. 1–5) the effect of "preconditioning" prior to immersion in water was examined. In tests 1 and 4 the specimens were bedded in moist sand overnight so that the surface of the stroma was level with the sand surface, thus allowing a slow uptake of moisture by the stroma via the wood

surface in contact with the sand. In tests 2, 3, and 5, the specimens were kept air-dry. After the overnight preconditioning period (12–16 hr) all specimens were immersed in water for the periods indicated, and tested for ascospore release in quadruple ascospore-liberating tunnels.

The second series (Table 3, Nos. 6–9) was an attempt to simulate the form of moisture uptake likely to occur in the field when dry weather ends with a sudden heavy rain. The surfaces of wood beneath each stroma were waterproofed with finger-nail lacquer, and the specimens then inverted with the surface of each stroma contacting a water surface level with the top of a van Tieghem ring sealed to a

TABLE 3

ASCOSPORE RELEASE FROM E. ARMENIACAE STROMATA IN LABORATORY TESTS

In first series of tests (Nos. 1–5) treatment was by immersion in water; in second series (Nos. 6–9) specimens treated by surface-wetting only

Test No.	Preconditioning Treatment	Period of Treatment (min)	Ascospore Release*	
			First Hour	Second Hour
1	16 hr in damp sand	20	+++	++
2	Air-dry	60	++	†
3	Air-dry	20	—	—
4	12 hr in damp sand	25	+++	++
5	Air-dry	25	±	±
6	Air-dry	30	±	—
7	Air-dry	60	++	±
8	Air-dry	90	+++	+
9	Air-dry	120	+++	+

* +++ Abundant release; ++ moderate release; + small release; — no release.
† No reading taken.

microscope slide. By this means, the stroma received moisture only via its outer surface. After various intervals of surface-wetting, specimens were transferred to the spore-liberating tunnels for 2 hr and tested at an air temperature of 13–14°C.

Non-uniform deposition of the ascospores on the trap slides, believed to be a combined result of the shape of the source material and the relatively small amount of lateral diffusion between source and trap in the spore-liberation tunnels, made counting impracticable. The amount of ascospores trapped was therefore assessed visually by a rapid microscopic examination of the whole area of deposit on each trap slide and allotted to one of the four categories shown in Table 3. Each test comprised at least two replicates, but individual results from replicates are shown only for those tests (Nos. 5, 6, and 7) in which there was inconsistency.

IV. DISCUSSION

The data of Section II show that stromata of *E. armeniacae* clearly have a very long life span. The limited histological studies have revealed that a stroma is productive for more than one year, and that a new crop of perithecia develops during

winter between the exhausted perithecia of the previous year. Further work is required, however, to determine whether perithecium production continues throughout the dispersal season each year (September–April) or whether ascospore production is limited to those perithecia which are mature by early spring (September). It would also be interesting to know the extent of area increase of individual stromata each year.

From the data of Table 3 we conclude that, in general, a preconditioning treatment which allows the wood beneath a stroma to imbibe water, thus becoming a reservoir of moisture for the stroma, results in a longer and more abundant ascospore release than occurs when an air-dry stroma is wetted only through its surface. This situation may be likened to conditions in the field when a sudden heavy shower of rain falls on stromata whose substrate has been in contact with damp soil or wet vegetation. Abundant and prolonged ascospore release occurred after 20-min immersion of an already-damp stroma (test No. 1) whereas a 30-min surface-wetting of a previously air-dry stroma produced either a small release of short duration or no release (test No. 6). Ascospore release comparable with that obtained after 20–25 min immersion of a damp specimen required more than 1 hr of surface-wetting of a previously dry specimen.

It is now apparent that ascospore production of *E. armeniacae* is at a maximum in spring, summer, and autumn. Summer rains may produce a high airborne concentration but the periods of ascospore release in summer are limited in South Australia by the low rainfall frequency. Knowledge of the annual periodicity now makes it possible to understand why Carter (1957*a*) failed to obtain natural infection of freshly made wounds exposed for intervals of 4 weeks during the winter of 1955.

The period of low ascospore output in 1962 and 1963 coincided closely with apricot tree dormancy in the non-irrigated districts of South Australia, extending from leaf-fall in late autumn to "pink-bud" in early spring. It seems reasonable to suppose that the coincidence between cycles of activity in host and pathogen has some biological significance, and one naturally seeks an explanation connected with survival of the pathogen. In search of such an explanation, work is now in progress to assess the deposition efficiency of *E. armeniacae* ascospore octads on the various surfaces presented by foliated and defoliated apricot shoots.

The seasonal pattern of ascospore dispersal, evident from the studies here reported, suggested a need to re-examine current South Australian recommendations for pruning commercial plantings of apricot trees (Moller 1964*b*). Revised recommendations have now been published (Moller and Carter 1964).

V. Acknowledgments

We are indebted to Messrs. L. S. Plush & Sons (Light Pass) and Mr. I. A. Martin (Berri) who willingly assisted in the operation of spore traps and rainfall recorders on their properties.

Dr. W. H. English, University of California, was associated with the studies reported in Section III(*d*).

VI. References

Carter, M. V. (1957*a*).—*Eutypa armeniacae* Hansf. & Carter, an airborne vascular pathogen of *Prunus armeniacae* L. in southern Australia. *Aust. J. Bot.* **5**: 21–35.

Carter, M. V. (1957*b*).—Vines aid spread of apricot "gummosis". *J. Dep. Agric. S. Aust.* **60**: 482–3.

Carter, M. V. (1960).—Further studies on *Eutypa armeniacae* Hansf. & Carter. *Aust. J. Agric. Res.* **11**: 498–504.

Carter, M. V. (1963).—*Mycosphaerella pinodes*. II. The phenology of ascospore release. *Aust. J. Biol. Sci.* **16**: 800–17.

Gregory, P. H. (1945).—The dispersion of air-borne spores. *Trans. Brit. Mycol. Soc.* **28**: 26–72.

Hirst, J. M. (1952).—An automatic volumetric spore trap. *Ann. Appl. Biol.* **39**: 257–65.

Hirst, J. M. (1953).—Changes in atmospheric spore content : diurnal periodicity and the effects of weather. *Trans. Brit. Mycol. Soc.* **36**: 375–93.

Hirst, J. M. (1959).—Spore liberation and dispersal. In "Plant Pathology : Problems and Progress, 1908–1958". (Ed. C. S. Holton.) pp. 529–38. (University of Wisconsin Press: Madison.)

Hirst, J. M., and Stedman, O. J. (1962).—The epidemiology of apple scab (*Venturia inaequalis* (Cke.) Wint.). II. Observations on the liberation of ascospores. *Ann. Appl. Biol.* **50**: 525–50.

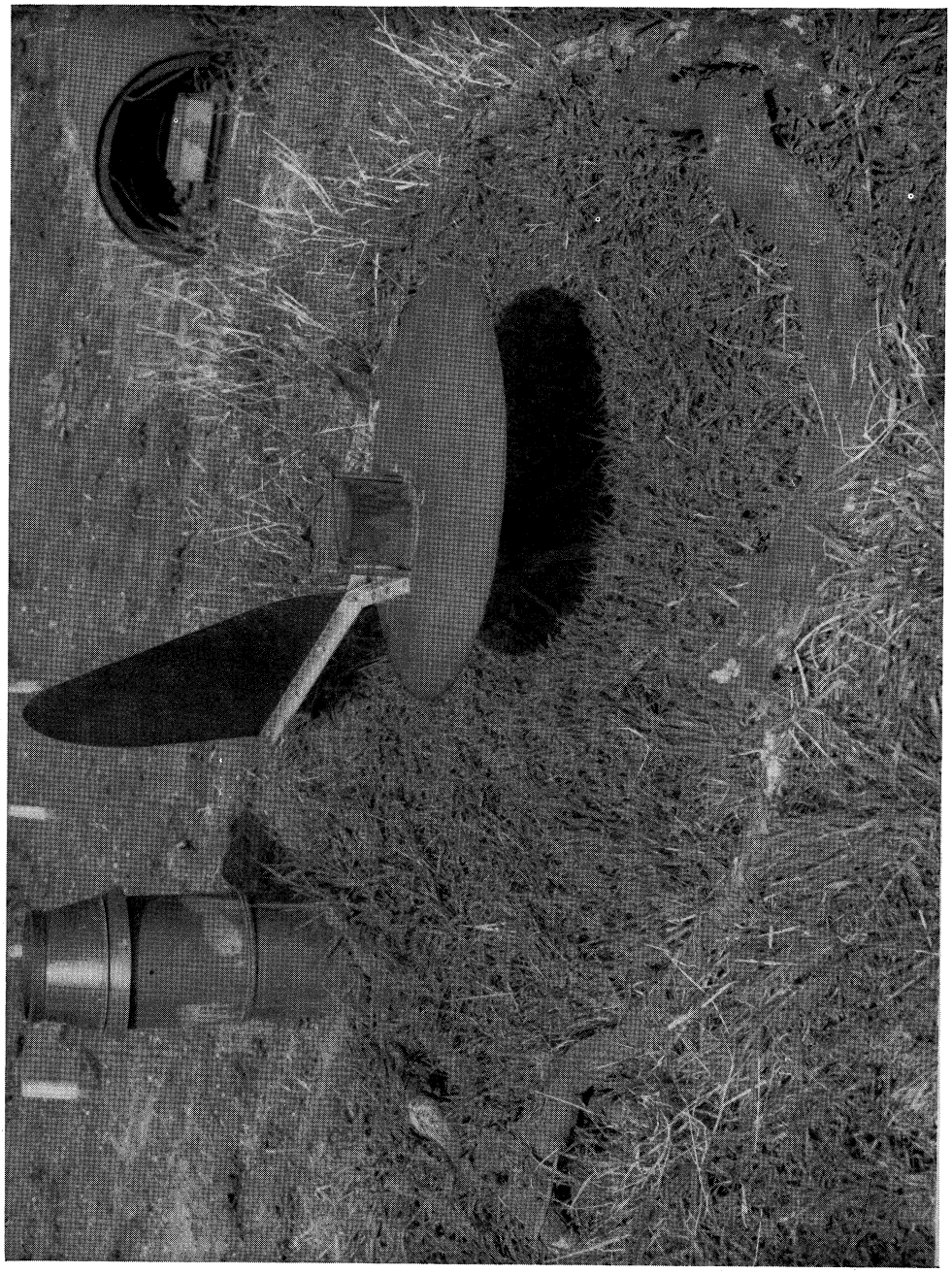
Ingold, C. T. (1960).—Dispersal by air and water—the take-off. In "Plant Pathology: An Advanced Treatise". Vol. 3. Ch. 5. pp. 137–68. (Eds. J. G. Horsfall and A. E. Dimond.) (Academic Press, Inc.: New York.)

Moller, W. J. (1964*a*).—Apricot disease found on garden shrub. *J. Dep. Agric. S.Aust.* **67**: 251.

Moller, W. J. (1964*b*).—The relationship between time of pruning and *Eutypa armeniacae* infection in apricots. Exp. Rec. Dep. Agric. S. Aust. No. 2.

Moller, W. J., and Carter, M. V. (1964).—Prune in June — new slogan for apricot growers. *J. Dep. Agric. S. Aust.* **67**: 322–4.

PRODUCTION AND DISPERSAL OF ASCOSPORES IN EUTYPA ARMENIACAE



Spore trap at centre of ring source, and rainfall recorder, Waite Institute. Photograph by Mr. B. A. Palk.