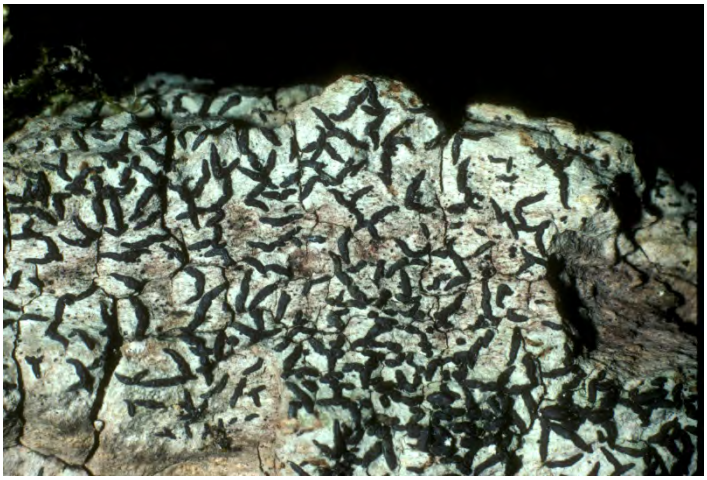
Seasonality of ascospore production in two lirellate lichens: *Alyxoria (Opegrapha) varia* and *Phaeographis dendritica*

Abstract: Ascospore liberation by *Alyxoria* (*Opegrapha*) *varia* in the field was maximal in March and November and negligible in summer. For *Phaeographis dendritica* it was maximal in winter but the total discharge was more than an order of magnitude lower than that of *Alyxoria* at the same locality. *Alyxoria* spore liberation was negatively correlated with temperature suggesting that prolonged wetting of apothecia in the colder months stimulated dispersal.



Alyxora (*Opegrapha*) *varia*, one of the species studied

The majority of lichenized fungi belong to the *Ascomycota*, most of which develop fruit bodies (ascomata) to a greater or lesser extent. Ascospore dispersal mechanisms are similar to those occurring in the non-lichenised forms and are wide-ranging. In some genera, ascospores are exuded onto the surface of the ascoma where they may be dispersed by invertebrates or water. However, for the majority they are launched explosively into the air where they are dispersed by wind (Pyatt, 1974; Bailey, 1976). The dispersal of ascomycete spores is a topic of some importance since in many cases they appear to offer the only means of propagation. The diversity of ascoma types, ascopores and asci, the sac-like bodies in which the spores develop, must reflect upon the dispersal mechanism since this is ultimately the reason for their existence. Despite this, dispersal mechanisms for lichenised species, including the mechanics of spore liberation, the energy invested in their dispersal, and the seasonality of spore liberation have received little study. Bailey (1976) reviewed the earlier literature on lichen ascospore dispersal, but there has been little research on the topic since. More recent work has focused upon spore size and dispersal rather than spore liberation (e.g. Tibell, 1994; Smith, 1995). This article describes the results of a one-year investigation into the *in vivo* liberation of ascospores by two common crustose lichens, *Phaeographis dendritica* and *Alyxoria* (*Opegrapha*) *varia*. Although phylogenetically distant, these taxa occur in similar habitats and both possess elongate ascomata known as lirellae.

Methods

This image shows the ash tree where the *Alyxoria* was studied. It is in a sheet on the lower part of the trunk. This tree is interesting as it also supports *Lobaria pulmonaria*, one of very few trees in SE England to do so. The *Phaeographis* site is also visible. It is among the small shrubby trees just behind and to the right of the ash tree.

The lichens were investigated in the ancient woodland of Eridge Old Park, East Sussex, UK (51º 06'N 0º 14'E; Nat grid ref. 51/577335; altitude 110 m). *Alyxoria varia* colonised the vertical bole of an ancient ash adjacent to a small stream. This site was used previously to study ascoma fertility (Pentecost, 2014). *Phaeographis dendritica* grew on the stems of a young oak 10 m to the north. To observe ascospore liberation, glass microscope slides were mounted over thalli so that their surface was approximately 2 mm distant using locating pins attached to the bark 1-2 m above ground. This measurement was chosen as Garrett (1971) found that most lichen ascospores were discharged to distances >2mm from apothecia. Slides were thinly smeared with petroleum jelly and left in the field for one month after which they were removed for examination and a new slide put in its place. The operation was repeated over the course of a year. Slides were examined for ascospores under a light microscope at a magnification of 250x. Between 50 and 200 fields of view were examined and the number of spores counted converted to spores cm^{-2} slide surface. Measurements of relative humidity (whirling psychrometer), air and bark temperature (thermistor) and relative light intensity (EA22 Luxmeter) were made at monthly intervals. Rainfall and mean air temperature records were obtained from a weather station located at the same altitude 6 km to the north.

Results

Monthly ascospore liberation figures as revealed by the glass slides are shown in Figures 1 and 2. Discharge of *Alyxoria varia* ascospores was found to be strongly seasonal (Fig. 1) with maxima recorded in March and November. During summer,

discharge was negligible. Bark surface temperature ranged between 5.2 and 22.4 °C (Fig. 1, Table 1), while relative humidity showed wide variation. Relative light intensity fell to low values during summer when the trees were in full leaf.

Data for *Phaeographis dendritica* are shown in Fig. 2. While maximum discharge also occurred during winter, spores were observed throughout the summer but the total number deposited on the slides was more than an order of magnitude lower than *Alyxoria*. Microclimatic measurements indicated a similar environment to the *Alyxoria* (Fig. 2, Table 1). Bark surface temperature, relative humidity and relative irradiance were all marginally lower, but in no case were the differences statistically significant.

Mean air temperature and precipitation totals for the period of study are shown in Fig. 3. These data correspond to the sampling periods and show that the coldest and warmest periods were respectively November - December (4.2 °C) and July - August (17.6 °C). The annual mean air temperature was 10.5 °C. There was no strong seasonal trend in precipitation but the highest total (140 mm) was reported for October - November and the lowest for July - August (6 mm). Total annual precipitation was 767 mm. Statistical data relating ascospore discharge to climate are shown in Table 2. Two significant associations are apparent. *Alyxoria varia* ascospore deposition and bark surface temperature was correlated negatively suggesting that as temperature rose, deposition fell. Although the mean air temperature correlation appears as non-significant in the table, a weak negative association is apparent as the P value of 0.068 is close to the critical value of 0.05. The second significant association was between *Phaeographis dendritica* spore deposition rate and relative humidity, suggesting that as humidity rose, so did deposition.

Discussion

The data demonstrate that ascospore discharge *in vivo* is highly variable but correlated with environmental conditions. However, meaningful field measurements of discharge are difficult to obtain, since spore collection requires imposition of a recording surface that will alter to some extent the local environment of the lichen. Placing a glass slide close to the lichen surface restricts the wetting and drying of apothecia although the method should allow at least approximate estimates where sampling is undertaken over periods of a month or so. One of the more surprising outcomes was the large difference in the number of trapped ascospores between the two lichens. Although differences in ascospore production are to be expected, with lichens with similar ascomata and spore types, an order of magnitude difference was not expected since both thalli were abundantly fertile. One possibility is that the *Phaeographis* ascomata were ageing and becoming senescent and thus unable to release large numbers of spores.

Discharge of ascospores has previously been found to occur when the water content of ascomata changes (ie. wetting or drying). Several workers have suggested that spore liberation was more prevalent in spring and autumn but the data were often conflicting (Bailey, 1976). More recently, Favero-Longo *et al.* (2014) found that ascospore numbers of the *Teloschistaceae* collected from the air spora were correlated with rainfall events. While this could be fortuitous, the finding supports the positive

correlation between *Phaeographis* spore emission and relative humidity obtained here. However, spore numbers were low and the investigation needs to be repeated owing to the infrequency of the microclimate measurements.

Work by Bailey & Garrett (1968) with *Lecanora conizaeoides* suggested that spore discharge was not influenced by light although lower temperatures appeared to be more favourable to discharge. These observations support those obtained here. Lower temperatures however, are likely also to correlate with longer periods of wetting owing to a reduced evaporation rate, and this may stimulate greater ascospore release. Bailey & Garrett (1968) found that *L. conizaeoides* was capable of releasing ascospores at any time of year, but earlier work on *Xanthoria parietina* (Werner, 1927) suggested that winter and spring were the best times. Studies on other lichens by Verseghy (1965) and Pyatt (1969) support this, along with the work reported here. Further research is clearly needed to clarify the relationships between discharge and microclimate and extend the work to other lichen species.

Acknowledgements

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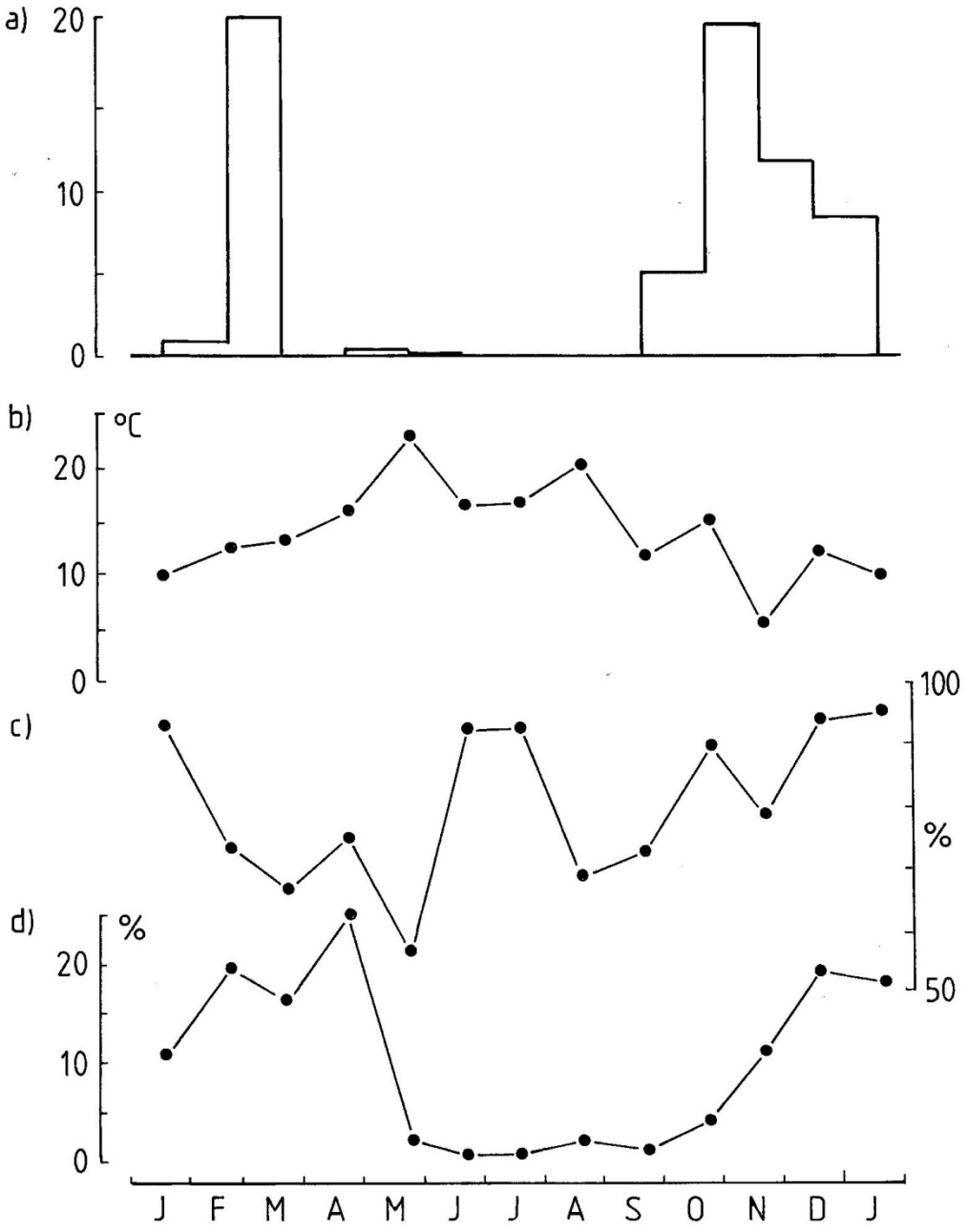

Figure 1. Monthly ascospore production and microclimate for *Alyxoria varia* during 1998-9.
a) Spore production
b) Bark surface temperature
c) Relative humidity
d) Relative light intensity compared with the open sky (100%)

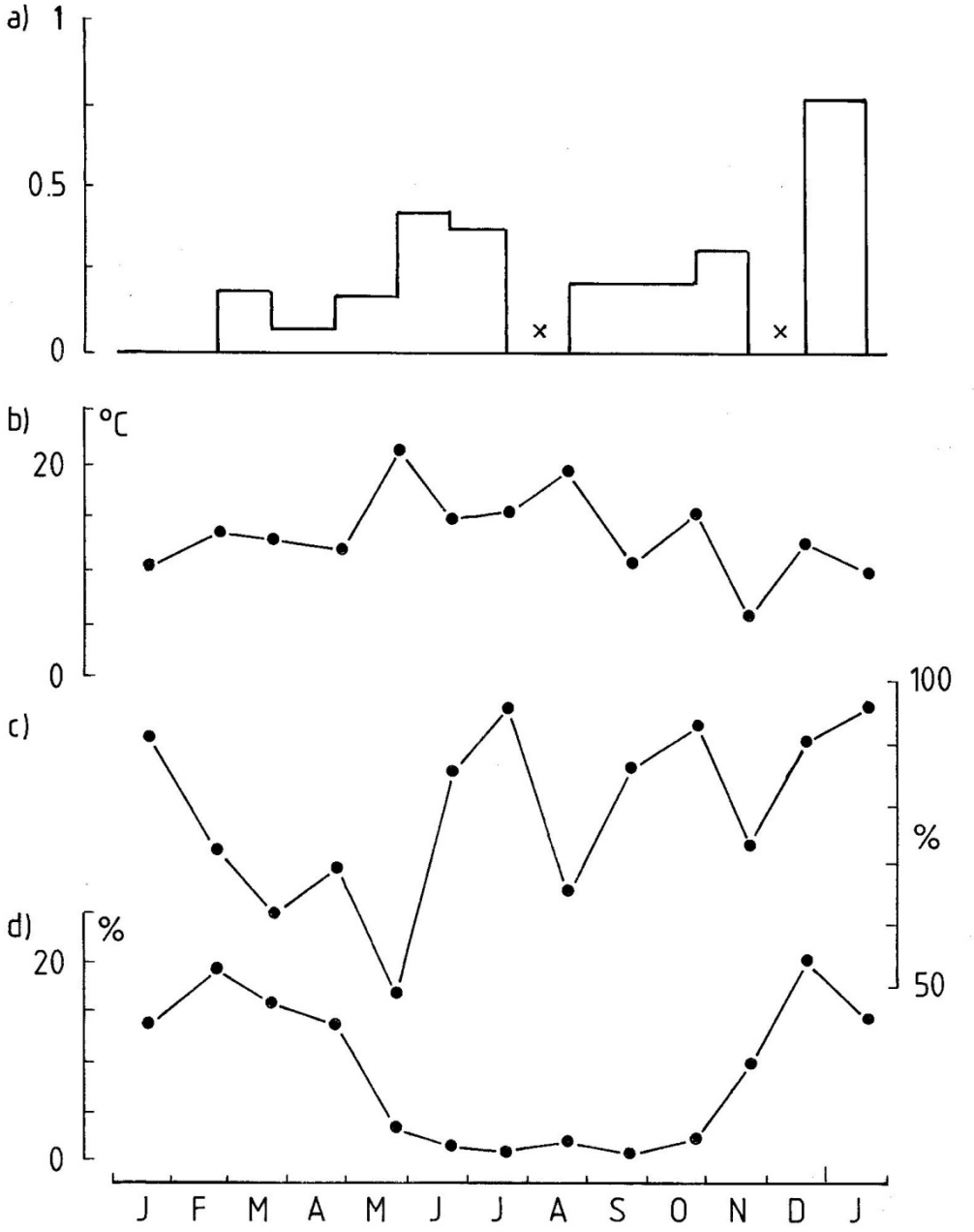

Figure 2. Monthly ascospore production and microclimate for *Phaeographis dendritica* during 1998-9. X denotes lost slide.
a) Spore production
b) Bark surface temperature
c) Relative humidity
d) Relative light intensity compared with the open sky (100%)

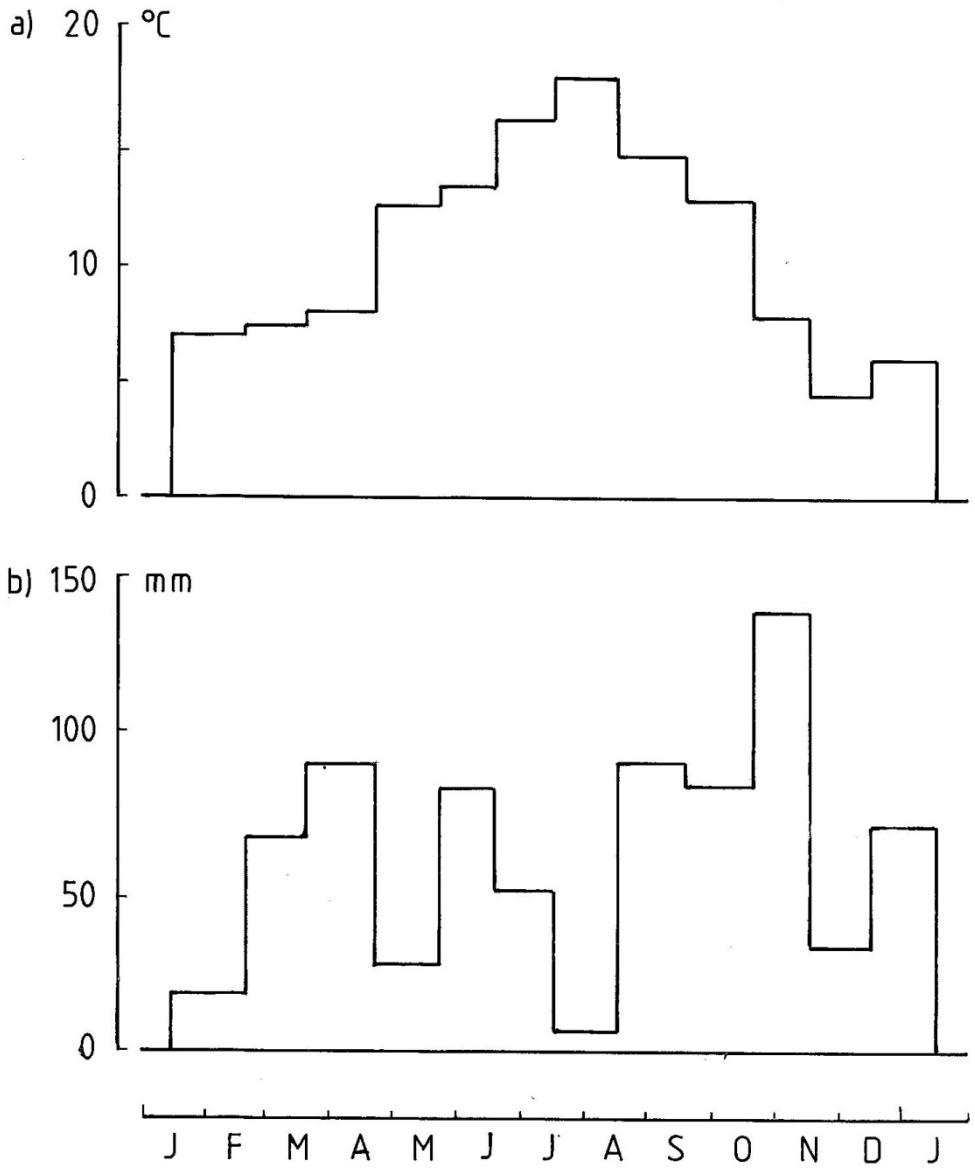

Figure 3. Mean monthly air temperature (a) and precipitation totals (b) taken from a nearby weather station. Measurements correspond to the 12 sampling periods.

Table 1. Summary microclimate data for the two sites, with means and (range).

	Bark surface temperature °C	Relative humidity %	Relative light intensity %
Alyxoria varia	13.7 (5.2-22.4)	80 (55-95)	10.0 (0.7-25)
Phaeographis dendritica	13.3 (5.4-21.4)	79 (48-96)	8.9 (0.4-21)

Table 2. Pearson product-moment correlation coefficients between ascospore deposition and climatic measurements. ns: no significant difference at p=0.05

	Mean air temperature	Bark surface temperature	Precipitation	Relative humidity	Relative light intensity
Alyxoria varia	-0.57 ns	-0.71*	0.5 ns	0.02 ns	0.32 ns
Phaeographis dendritica	-0.02 ns	-0.25 ns	0.23 ns	0.68*	-0.16 ns

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