Plant Pathology (1995) **44**, 662–674

Effects of humidity, leaf wetness, temperature and light on conidial production by *Phaeoisariopsis personata* on groundnut

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Controlled-environment studies of conidial production by *Phaeoisariopsis personata* on groundnut are described. With constant relative humidity (RH), conidia were only produced above a threshold (94·5% RH) and there was a linear increase between 94·5% RH and 100% RH. Conidial production was less with continuous leaf wetness (resembling heavy dew) than with continuous 98–99% RH, but it was similar with intermittent leaf wetness and intermittent 98–99% RH (8 h at 70% RH each day). With alternate high ($\geqslant$97% RH) and low humidity, daily conidial production depended both on the duration of high RH and on the low RH value. With 99% RH at night (12 h), night-time conidial production decreased with the previous daytime RH. After conidial production had started, small numbers of conidia were produced even when the RH was well below the threshold (94·5%). Conidia were produced in continuous light when the photon flux density was 2 μmol/m^2/s, but production was completely inhibited with 60 μmol/m^2/s. With constant RH, more conidia were produced with a 12 h photoperiod than in continuous darkness. However, more than 75% of the conidia were produced in the dark. With continuous darkness, more conidia were produced during the night (18.00–06.00 h) than during the day, but this biological rhythm was overcome with a (light-night)/(dark-day) regime. With constant 98–99% RH there was a linear increase in conidial production with temperature between 10 and 28°C, and virtually no conidia were produced at 33°C. The daily production of conidia increased with time for 2 to 6 days, depending on the treatment.

INTRODUCTION

Late leaf spot of groundnut (*Arachis hypogaea* L.), caused by *Phaeoisariopsis personata*, is widespread wherever the crop is grown, and commonly causes pod losses of 10 to 50% (Smith *et al.*, 1992). The disease is normally spread by conidia which are dispersed by wind, splashing water or insects (Smith, 1984).

Leaf wetness is necessary for infection (Butler *et al.*, 1995), and the effects of temperature and leaf wetness periods have been quantified (Butler *et al.*, 1994). After the appearance of initial lesions in the crop, sporulation is necessary for polycyclic disease development, and the extent of sporulation affects the rate of disease increase. Sporulating lesions can be identified by the presence of caespituli (swellings), usually on the abaxial leaf surface, and we observed in latent period studies that the caespituli do not erupt to release conidia until the humidity is close to saturation (Wadia & Butler, 1994a). A single conidium at a time is produced on each conidiophore and, prior to detachment, the conidiophore resumes growth subterminally (Brown & Brotzman, 1979). Conspicuous conidial scars are left after detachment from the conidiophore (Deighton, 1967).

With *Cercospora* species, leaf wetness, humidity, temperature and light have important effects on conidial production (e.g. Cooperman & Jenkins, 1986; Alderman & Beute, 1987; Carisse *et al.*, 1993), but we have not found any reports quantifying similar effects for *P. personata*. The present paper describes a series of controlled-environment experiments aimed at determining the effects of humidity, leaf wetness, temperature and light on conidial production by *P. personata*.

MATERIALS AND METHODS

Plant material

Experiments were carried out on the groundnut cultivar TMV 2, which is susceptible to late leaf

spot disease. Potted plants were raised in a glasshouse using a potting medium composed of 50% loam, 25% sand and 25% compost. The medium was steam sterilized and the plants were supplied with Broughton's nutrient solution (Broughton & Dilworth, 1971). The glasshouse temperature did not exceed 35°C and the minimum temperature varied between 12 and 20°C. The minimum relative humidity (RH) ranged from 30 to 50% during the day and from 60 to 90% during the night.

Inoculum and inoculation

Conidia of *P. personata* were multiplied by inoculating groundnut leaves (cv. TMV 2) with inoculum derived from locally collected conidia using the method described by Wadia and Butler (1994a). The conidia were harvested with a cyclone spore collector and stored at 4°C. For sporulation experiments, 4-week-old plants (one per pot) were inoculated by spraying an aqueous suspension of 1000 conidia/ml with an atomiser (Wadia & Butler, 1994b). To ensure infection, intermittent leaf wetness was provided by transferring plants to a dew chamber (Butler *et al.*, 1994) set to 23°C for 5 nights (16 h per night), and moving them each day to a glasshouse or to a plant growth cabinet (E15 Conviron, Winnipeg, Canada) at 26°C, 70% RH and 450 μmol/m^2/s.

After providing intermittent leaf wetness for 5 days, the plants were incubated in the glasshouse for a further 15–16 days. The inoculum concentration of 1000 conidia/ml resulted in small numbers (less than 1·5 lesions/cm^2 leaf area) of circular lesions. For each experiment, five lesions of uniform size were marked on each plant and their diameters were measured with a ruler at the beginning and end of the experimental period. Where possible, selected lesions were well separated from each other and were not adjacent to the leaf margin.

Spore collection

Conidia were collected non-destructively from marked lesions, returning to the same lesion repeatedly during each experiment, and using a spore sampler modified from the design of Woolacott & Ayres (1984). The modifications included a straight input tube (4 mm internal diameter) with spacers to maintain a constant distance of 1 mm between the end of the tube and the leaf surface. The sampler was connected to a vacuum pump which provided an air flow of about 15 l/min. Conidia were impacted directly onto a coverslip coated with a thin film of petroleum jelly mixed with wax. A standard procedure was adopted, which involved holding the inlet tube over each lesion and operating the vacuum pump for 20 s. A new coverslip was used for each lesion, and the input tube was cleaned by swabbing with alcohol between treatments.

In order to test the efficiency of the spore sampler, we repeatedly collected conidia from the same lesion (for 20 s each occasion) and demonstrated that, on average (the mean of 10 determinations), 80% of the conidia were collected during the first 20-s period. No conidiophores were removed by the spore sampler.

The coverslips were mounted in glycerine on a microscope slide and were examined at ×66 magnification in order to count the conidia collected from each lesion. The number of conidia in each sample was expressed per unit lesion area, assuming lesions to be circular and estimating their areas from their diameters. Between the beginning and end of each experiment lesion diameters were interpolated linearly. The validity of the linear interpolation was tested by measuring the diameter of 40 lesions each day to an accuracy of 0·01 mm (Mitutoyo Digimatic Digital Caliper, RS Components, Corby, UK) over an 8-day period. The result was examined graphically.

Experiments

In order to examine the effects of humidity on conidial production, single-plant controlled-humidity chambers (Butler *et al.*, 1995) were used. Relative humidity was controlled with a data logger (21X, Campbell, Logan, UT, USA) which had been programmed with the desired RH value, hereafter referred to as the 'set value'. The relative humidity achieved was stable to within ±0·5% RH and was always within 1% RH of the set value. These chambers were located in a room where a stable temperature close to 26°C was maintained and there was no natural light.

In order to examine the effects of leaf wetness and temperature on conidial production, dew chambers (Butler *et al.*, 1994) were used. Each dew chamber consisted of an outer cabinet and an inner chamber (containing the plants) with a heated water bath. Substantial leaf wetness (resembling heavy dew) was achieved by maintaining the outer cabinet at about 10 K less than the inner chamber. High RH (98–99%) with

Table 1. Details of experiments with different night/day humidities

Experiment number	High RH		Low RH		Collection frequency of conidia (h)	Duration of experiment (days)	Repetition of experiment
	Set value (%)	Period (h)	Set value (%)	Period (h)			
1	100	18.00–06.00	100	06.00–18.00	24	8	1
	97	18.00–06.00	90	06.00–18.00	24	8	1
	97	18.00–06.00	70	06.00–18.00	24	8	1
	97	18.00–06.00	50	06.00–18.00	24	8	1
2	100	18.00–06.00	100	06.00–18.00	12	6	0
	99	18.00–06.00	90	06.00–18.00	12	6	0
	99	18.00–06.00	70	06.00–18.00	12	6	0
	99	18.00–06.00	50	06.00–18.00	12	6	0
3	100	18.00–06.00	100	06.00–18.00	24	8	1
	99	18.00–06.00	92	06.00–18.00	24	8	1
	99	18.00–06.00	90	06.00–18.00	24	8	1
	99	18.00–06.00	80	06.00–18.00	24	8	1
4	100	18.00–06.00	100	06.00–18.00	12	6	0
	99	18.00–06.00	92	06.00–18.00	12	6	0
	99	18.00–06.00	90	06.00–18.00	12	6	0
	99	18.00–06.00	80	06.00–18.00	12	6	0
5	100	18.00–06.00	100	06.00–18.00	24	8	1
	99	18.00–06.00	80	06.00–18.00	24	8	1
	99	22.00–06.00	80	06.00–22.00	24	8	1
	99	00.00–04.00	80	04.00–24.00	24	8	1

little or no leaf wetness was achieved by maintaining the outer cabinet at 1–2 K less than the inner chamber. The humidity in the inner chamber was measured with an aspirated wet-and-dry bulb psychrometer, with thermistor sensors calibrated individually to within ±0·05°C. The temperatures of the inner chambers were controlled by means of a data logger (CR10, Campbell, Logan, UT, USA).

Constant humidity

Two experiments were carried out at constant humidity.

In the first experiment, one plant was placed in each of four controlled-humidity chambers. One chamber was set to 100% RH and the set values in the other three chambers were between 92 and 99% RH. These settings were maintained for 5 days and conidia were collected every 24 h. The experiment was repeated 10 times, each time maintaining 100% RH in one chamber and selecting different set values in the remaining three chambers. Two 36-W fluorescent tubes, which provided a photon flux density of about 2 μmol/m^2/s in the chambers, were left on continuously.

In the second experiment, one plant was placed in each of four controlled-humidity chambers. Constant set values of 95, 97, 99 and 100% RH were maintained for 5 days and conidia were collected every 12 h. Two 36-W fluorescent tubes, as described above, provided a 12-h photoperiod (from 06.00 to 18.00 hours).

Leaf wetness vs. high RH

Two dew chambers were used, one of which was set to wet the leaves completely, while the other was set to give 98–99% RH with little or no leaf wetness. There were four treatments:

(1) continuous leaf wetness for 3 days;
(2) continuous high RH for 3 days;
(3) intermittent leaf wetness for 3 days
(4) intermittent high RH for 3 days.

In the case of intermittent treatments, plants were kept in the dew chambers for 16 h/day and transferred to a plant growth chamber at

70% RH for 8 h/day. For all treatments, a temperature of 23 C and dark conditions were maintained throughout (in both the dew chambers and the plant growth chamber). Conidia were collected from marked lesions 24, 48 and 72 h after the beginning of the experiment. The experiment was repeated once.

Different night/day humidities

Five experiments were performed in order to examine the effect of different combinations of night and day humidities. In all experiments, four of the controlled-humidity chambers referred to above were used. There were three treatment chambers and one 'control' with constant 100% RH. In all five experiments, a 12 h photoperiod (2 μmol/m^2 s) was provided from 06.00 to 18.00 hours. The treatments are summarized in Table 1.

In experiment 1, the set values were 97% RH at night (12 h duration), the three daytime values were 90, 70 and 50% RH, and conidia were collected at 24-h intervals (at 13.00 hours). In experiment 2, the set values were 99% RH at night, the three daytime values were the same

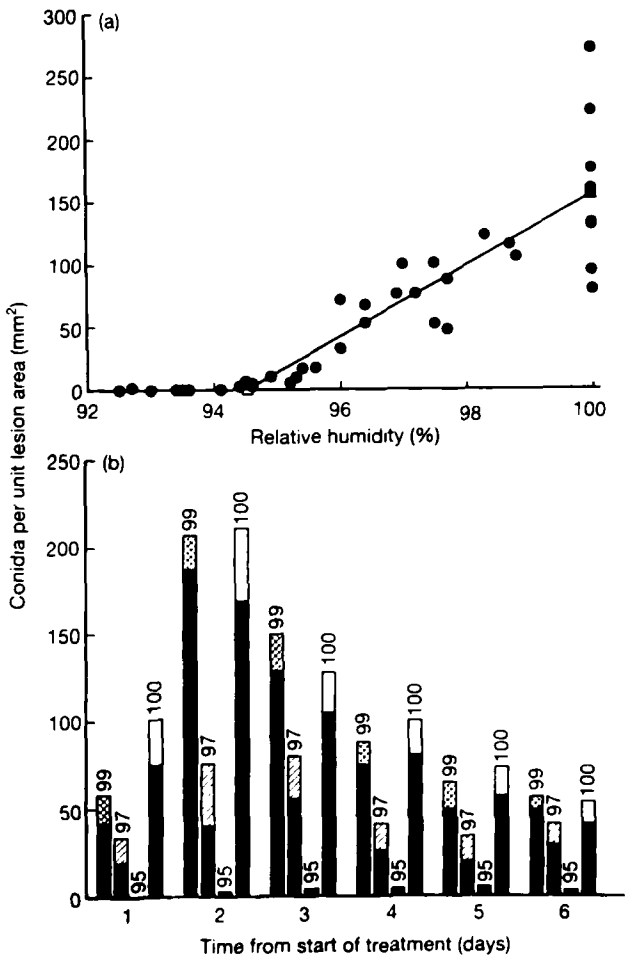

Fig. 1. Conidial production by *Phaeoisariopsis personata* with constant humidity at 26 C. (a) Mean number of conidia produced in 24 h with continuous light (2 μmol m^2 s) on days 3, 4 and 5, the linear regression ($y = 28.4x - 2684.4$; $r^2 = 0.77$) did not include values < 94% RH. (b) Conidia were collected every 12 h. Each bar represents the total number in 24 h; solid areas denote conidia collected at the end of the dark periods (06.00 hours) and open or hatched areas denote conidia collected at the end of the light periods (18.00 hours)

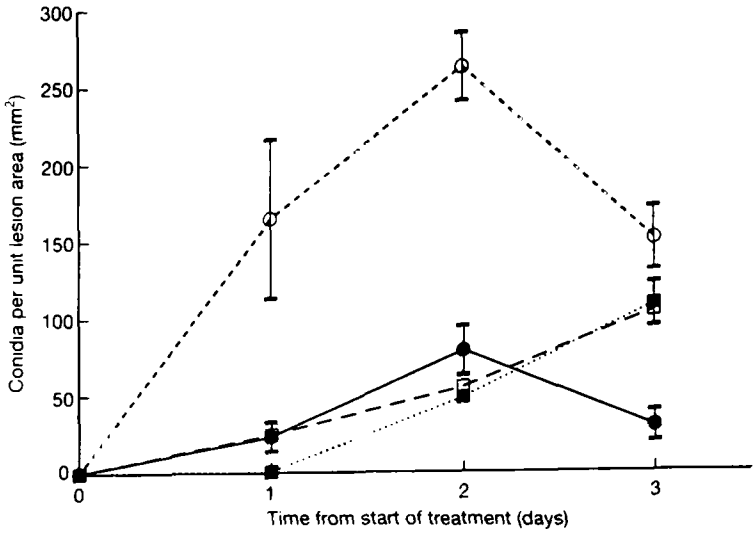

Fig. 2. The effects of leaf wetness and high RH (98–99%) on conidial production by *Phaeoisariopsis personata* at 25 °C. Conidia were repeatedly collected from the same lesions every 24 h: ---O---, continuous high RH; —●—, continuous leaf wetness; - - -□- - -, intermittent high RH (16 h/day); · · ■ · ·, intermittent leaf wetness (16 h/day).

as in experiment 1, but conidia were collected at 12-h intervals (at 06.00 and 18.00 hours).

In experiments 3 and 4, set values were 99% RH at night (12 h duration) and the three daytime values were 92, 90 and 80% RH for both experiments. Conidia were collected at 24-h intervals (at 13.00 hours) in experiment 3 and every 12 h (at 06.00 and 18.00 hours) in experiment 4.

In experiment 5, the number of hours of high low RH were varied within each 24-h period. The set values for all treatments were 99% RH (high) and 80% RH (low). Conidia were collected at 24-h intervals (at 13.00 hours).

Light

Three experiments were performed in order to examine the effect of light dark on conidial production.

In the first experiment, two plants were covered with clear polythene bags and placed in the growth cabinet at 25 °C. Lights (fluorescent tubes and incandescent bulbs, giving 60 μmol/m^2 s) were switched on from 06.00 to 18.00 hours for the (light-day) (dark-night) treatment. One of the plants was covered with thick black cloth in addition to the polythene, in order to maintain continuous darkness. The experiment continued for 5 days and the covers were removed briefly at 06.00 and 18.00 hours each day while conidia were collected.

The second experiment was similar to the first except that the lights were switched on from 18.00 to 06.00 hours for the (dark-day) (light-night) treatment. The continuous dark treatment was repeated and conidia were sampled at the same times as before.

In the third experiment there were two treatments: continuous light and continuous darkness. The other conditions were similar to those described for the first two experiments.

Temperature

Five dew chambers were set to give 98–99% RH with little or no leaf wetness. Each was set to a different temperature in the range 10 to 33 °C and one plant with five marked lesions was placed in each chamber in the dark. The experiment lasted for 5 days and plants were removed at 24-h intervals and kept in the laboratory for 30 min for the leaves to dry before conidia were collected from the marked lesions. The procedure was repeated three times with different temperature settings in the same range (10 to 33 °C).

Data analysis

The mean and standard error of the number of conidia per unit lesion area was calculated for each treatment (using counts from the five

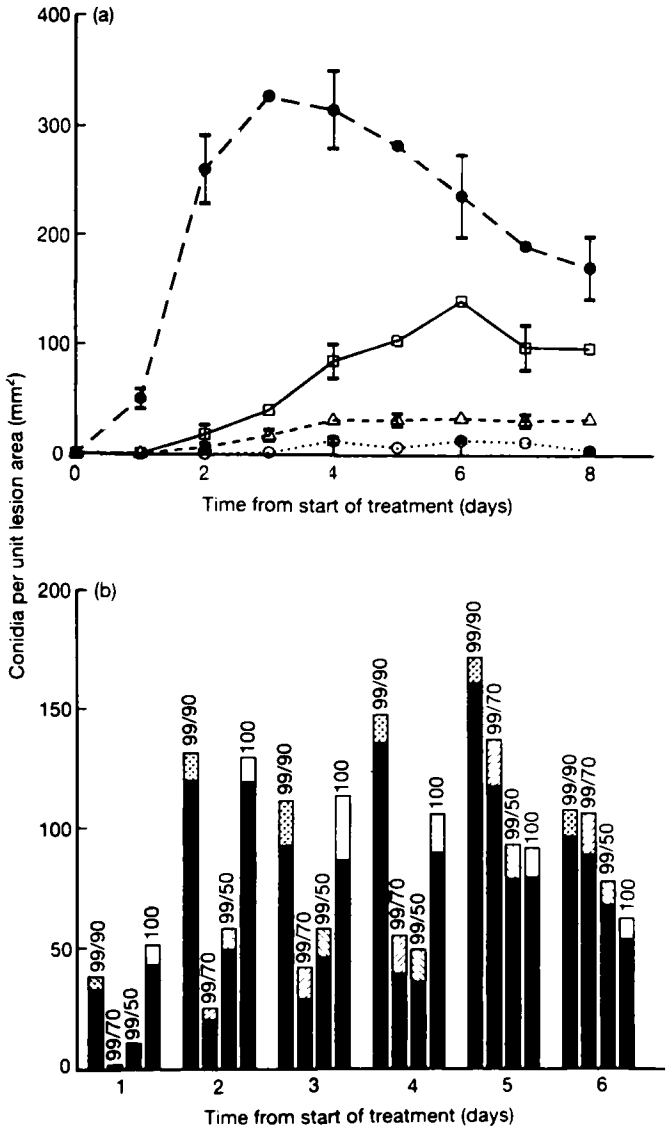

Fig. 3. Conidial production by *Phaeoisariopsis personata* with different night day humidity regimes at 26 C. (a) Conidia were collected every 24 h: -●- - -, 100/100% RH; —□—, 97 90% RH; ---Δ---, 97 70% RH, · O · ·, 97 50% RH. (b) Conidia were collected every 12 h. Each bar represents the total number in 24 h; solid areas denote conidia collected at the end of the dark periods (06.00 hours) and open or hatched areas denote conidia collected at the end of the light periods (18.00 hours).

marked lesions). Treatment means were compared using Student's *t*-test.

RESULTS

Constant humidity

In the first experiment (92–100% RH and continuous light with a photon flux density of 2 μmol m^{-2} s^{-1}), the number of conidia produced in 24 h increased with time for the first 3 or 4 days in all treatments and decreased on day 5. The average number of conidia collected on days 3 to 5 was used to represent the daily conidial production, and this increased linearly between 94·5% RH (the threshold humidity) and 100% RH (Fig. 1a).

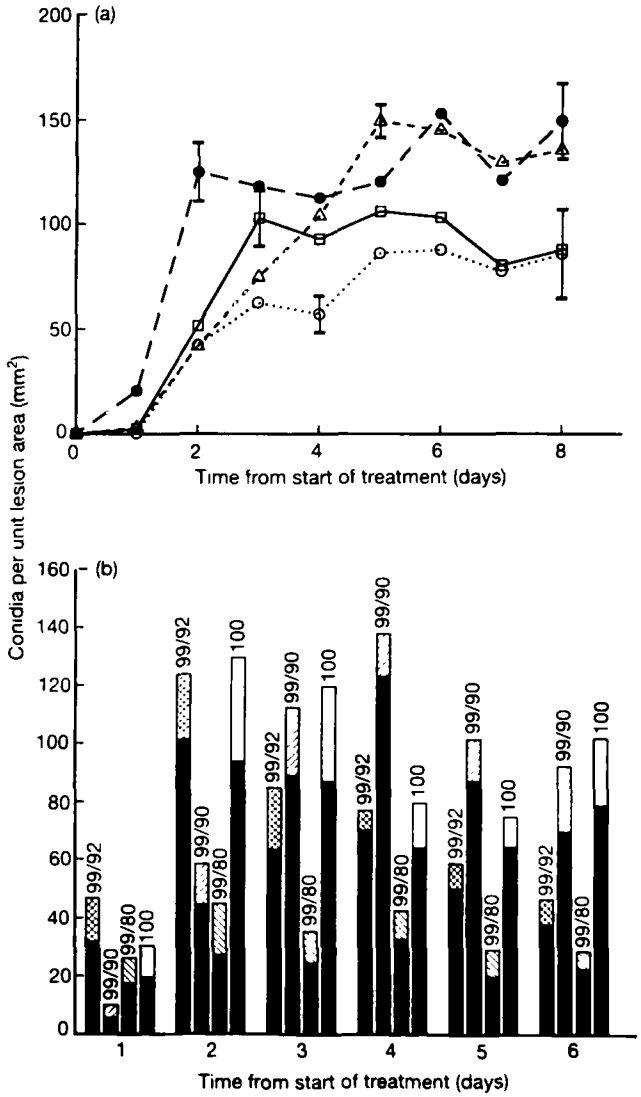

Fig. 4. Conidial production by *Phaeoisariopsis personata* with different night day humidity regimes at 26 C (a) Conidia were collected every 24 h: -- ● --, 100/100% RH; —□— 99 92% RH, ---Δ---, 99 90% RH, · ·O· ·, 99 80% RH. (b) Conidia were collected every 12 h. Each bar represents the total number in 24 h; solid areas denote conidia collected at the end of the dark periods (06.00 hours) and open or hatched areas denote conidia collected at the end of the light periods (18.00 hours).

In the second experiment (95–100% RH and 12-h photoperiod), the number of conidia collected at 06.00 hours (the end of the dark period) was consistently greater than at 18.00 hours (the end of the light period) (Fig. 1b). There was a sharp increase in the number of conidia collected between days 1 and 2, and subsequently the number decreased with time. There was generally little difference between the numbers of conidia in the 99 and 100% RH treatments, but there was a significant ($P < 0.001$) decrease between 99 and 95% RH, consistent with the first experiment.

Leaf wetness vs. high RH

Maximum numbers of conidia were collected

from lesions subjected to continuously high RH (98–99%), and this treatment resulted in four to five times more conidia than the continuous leaf wetness treatment (Fig. 2). The difference was highly significant ($P < 0.001$). In both these treatments maximum numbers of conidia were collected after 48 h.

The results obtained for the intermittent leaf wetness and intermittent high RH treatments were similar, and the numbers of conidia increased with time throughout the experiment.

Different night/day humidities

Experiment 1

Substantially fewer conidia were collected from the 97/90% RH regime than from the continuous 100% RH treatment (Fig. 3a), and the difference was highly significant ($P < 0.001$). Comparing all the treatments, as the daytime RH decreased the number of conidia also decreased significantly ($P < 0.001$).

Experiment 2

The number of conidia collected at the end of each dark period was much larger than the number collected at the end of each light period in all treatments (Fig. 3b). With the continuous 100% RH treatment, the maximum number of conidia occurred on day 2 and there was a subsequent decline. All other treatments reached a maximum number on day 5 and decreased on day 6. There were consistently and significantly fewer ($P < 0.001$) conidia in the 99/70% RH regime than in the 99/90% RH regime. Differences between the 99/70% RH and 99/50% RH regimes were not consistent, and were generally not significant.

Experiment 3

With the continuous 100% regime and the 99/92% RH regime, the number of conidia did not increase significantly after days 2 and 3, respectively (Fig. 4a). With the 99/90% RH regime, there was a gradual increase reaching a maximum on day 5, after which the number of conidia was significantly ($P < 0.001$) greater than in the 99/92% RH regime. The number of conidia in the 99/80% RH regime increased more gradually than in other treatments.

Experiment 4

In all treatments, the number of conidia was much higher at the end of each dark period than

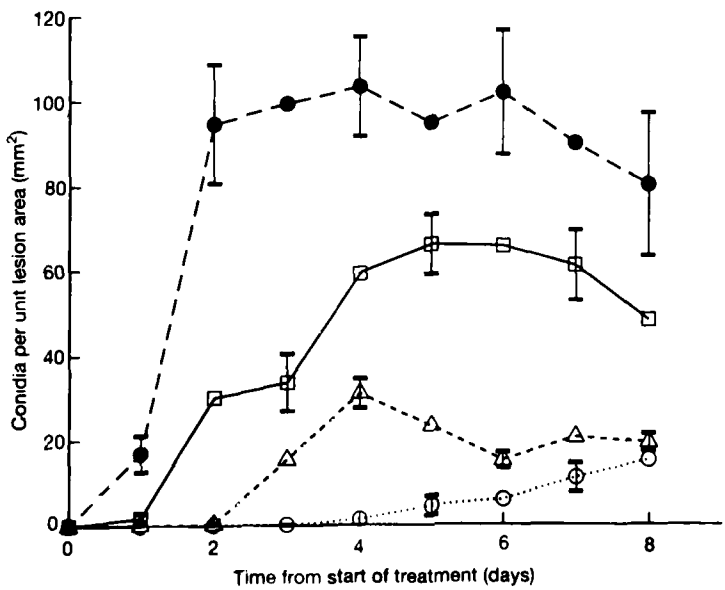

Fig. 5. Conidial production per 24 h by *Phaeoisariopsis personata* with 99/80% RH night/day humidity at 26 C: - ● -, continuous 100% RH (control); □ 12 h per day at 99% RH; ---Δ---, 8 h per day at 99% RH; ··○··, 4 h per day at 99% RH

at the end of each light period (Fig. 4b). With both the continuous 100% RH treatment and the 99/92% RH regime, maximum numbers of conidia were collected on day 2. The subsequent decline was most pronounced with the 99/92% RH regime. The number of conidia in the 99/90% RH regime reached a maximum on day 4, when it was significantly higher ($P < 0.01$) than the 100% RH control. The numbers of conidia collected in the 99/80% RH regime remained small throughout the experiment and were significantly less ($P < 0.001$) than for all the other treatments after day 2.

Experiment 5

The numbers of conidia collected for all treatments were consistently lower than the 100% RH control (Fig. 5). There were significantly fewer ($P < 0.001$) conidia in the treatment for 8 h at 99% RH than in the treatment for 12 h at 99% RH. With the treatment for 4 h at 99% RH, the number of conidia increased slowly and reached a similar level to that for the 8-h treatment on day 8.

Light

From days 2 to 5 there were significantly ($P < 0.001$) more conidia in the (light-day)/(dark-night) treatment than any other treatment, and more than 75% of the conidia were produced during the night (Fig. 6).

Similarly, in the continuously dark treatment consistently more conidia were produced at night (from 18.00 to 06.00 hours), but the total production was less than in the (light-day) (dark-night) treatment. Similar results were obtained for this treatment in all three experiments.

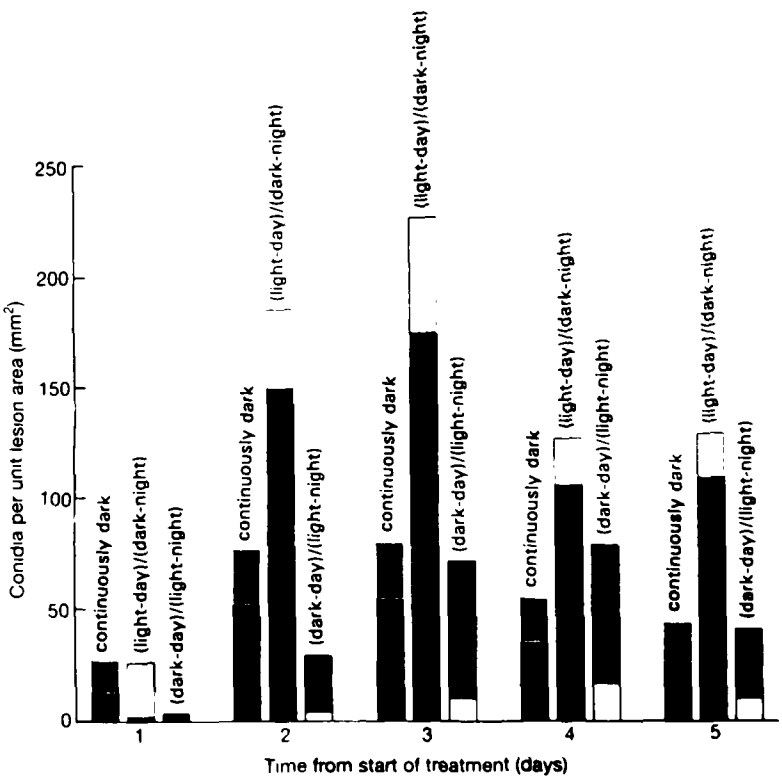

Fig. 6. Conidial production by *Phaeoisariopsis personata* with different light dark regimes. Plants were kept in polythene bags to maintain high RH, and the temperature was constant (25°C). Conidia were collected every 12 h. Each bar represents the total number in 24 h, with light/dark treatments, the solid areas denote conidia collected at the end of the dark periods and open areas denote conidia collected at the end of the light periods. With the continuous dark treatment, solid areas denote conidia collected at 06.00 hours and shaded areas denote conidia collected at 18.00 hours.

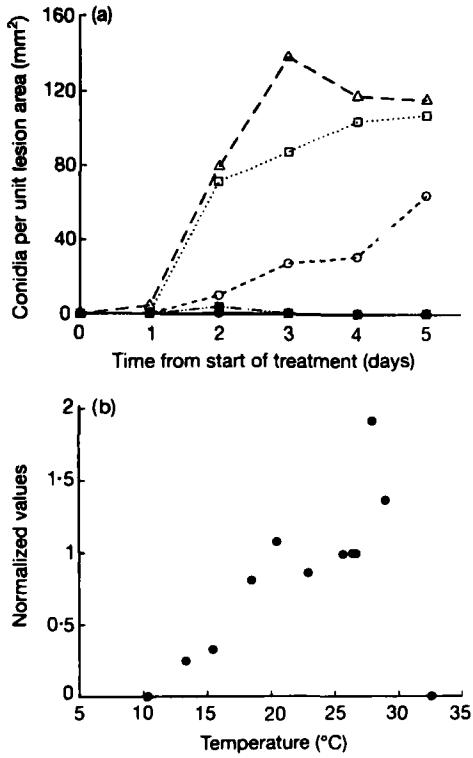

Fig. 7. The effect of temperature on conidial production by *Phaeoisariopsis personata*. (a) Change with time at different temperatures: –··–■–··–, 33°C; – – –△– – –, 26°C; ··□···, 21°C; ---○---, 15°C; —●—, 10°C. (b) Average number of conidia on days 3, 4 and 5.

In the (dark-day)/(light-night) treatment, the majority of conidia were produced in the dark (from 06.00 to 18.00 hours).

Continuous light (60 μmol/m^2/s) completely inhibited conidial production.

Temperature

At 26°C, the number of conidia reached a maximum after 3 days (Fig. 7a). At 21 and 15°C the number of conidia continued to increase throughout the experiment, and by day 4 the numbers at 21 and 26°C were similar. There were always significantly fewer ($P < 0{\cdot}001$) conidia at 15°C than at 21°C, and virtually no conidia were produced at either 10°C or 33°C.

The average number of conidia obtained on days 3, 4 and 5 was used to obtain a relationship between sporulation and temperature. Absolute numbers in the three experiments were different, so the values were normalized in each experiment by dividing the number of conidia per mm^2 in each treatment by the number at 26°C. The result (Fig. 7b) shows a virtually linear increase between 10 and 28°C, and a very sharp decrease between 28 and 33°C.

DISCUSSION

In the constant-humidity experiments, the threshold for conidial production was 94·5% RH. Similar humidity thresholds have been identified for sporulation by other pathogens, e.g. 92% RH for *Cercospora carotae* (Carisse *et al.*, 1993) and 93% RH for *Bipolaris* (*Helminthosporium*) *maydis* (Hyre, 1974). *Cercospora arachidicola* was observed to sporulate abundantly at 100% RH, but not at all at 90% RH (Melouk & Ketring, 1982). We found a linear increase in conidial production between the threshold and 100% RH for *P. personata* (Fig. 1a), compared to a logarithmic increase for *B. maydis* between 93% and 98% RH (Hyre, 1974).

Microscopic examination of lesions showed that the cuticle was intact over caespituli at the start of our experiments. When detached leaves were exposed to saturated humidity at 25°C, the caespituli erupted after 4–6 h. With constant high humidity, it took 2 days to reach the maximum conidial production per 24 h. Once caespituli had erupted and conidial production had started, further conidia were produced even when the RH was less than the threshold. This can be seen in Fig. 3b, where similar (small) numbers were collected at the end of the light periods at all humidities (from 50% to 100% RH). Furthermore, the caespituli erupted when conidial production was inhibited by continuous light (60 μmol/m^2/s), so it is likely that high RH is more important for caespituli eruption than for conidial production *per se*.

In constant conditions, similar numbers of conidia were produced at 99 and 100% RH (Fig. 1b), but fewer conidia were produced with 'heavy dew' than with high RH (Fig. 2). A similar situation was found with *Exserohilum* (*Helminthosporium*) *turcicum*, with more spores produced with continuous (36 h) high RH than with leaf wetness (Levy & Cohen, 1980). However, our results showed similar numbers of conidia with alternate periods of high and low RH and alternate leaf wetness and low RH (Fig. 2). Carisse *et al.* (1993) observed more *C. carotae* conidia with leaf wetness than at 96% RH, but they did not report the effect of higher RH values compared to leaf wetness. The high variability in spore production at 100% RH shown in Fig. 1a could have been caused by varying amounts of condensation on the leaves.

Conidia were produced with continuous light when the photon flux density was 2 μmol/m^2 s (Fig. 1a). However, conidial production was inhibited with a photon flux density of 60 μmol/m^2/s and, after 3 days of continuous light, conidiophores had developed (observations were made with a binocular microscope). Similar examples have been reported where spore differentiation was inhibited by light but sporophore formation was thought to be promoted (e.g. Nelson & Tung, 1973; Turian, 1974; Cooperman & Jenkins, 1986). In the case of *P. personata* we found more conidia produced with a light/dark regime (12/12 h) than with continuous darkness (Fig. 6). This could be due to the fact that conidiophores developed during the light and conidia were (mainly) produced in the dark. With continuous darkness, about twice as many conidia were produced during the night (18.00 to 06.00 hours) as during the day (06.00 to 18.00 hours). This indicates that part of the difference in conidia production between day and night is caused by a biological rhythm. This effect is reinforced when lights are operated during the day, but the biological rhythm is overcome with a (dark-day)/(light-night) regime (Fig. 6). Chen *et al.* (1979) observed that darkness inhibited sporulation of *Cercospora kikuchii* at 13°C, but that there was no effect at 22–28°C. We did not determine whether the effect of light was temperature dependent.

With constant humidity, light days and dark nights, maximum numbers of conidia were consistently produced on day 2, followed by a decrease. The decline in the number of conidia per unit lesion area resulted from an increase in lesion area; conidial production per lesion remained fairly constant. With different night/day humidities, however, conidial production continued to increase for a longer period, and production in the 99/90% RH regime commonly exceeded that in the control (continuous 100% RH) after day 3. A build-up of spore production with time has been observed with other pathogens. For example, in *Botrytis squamosa*, conidial production reached a peak 4 days after exposure to constant high RH and a 12/12 h light/dark regime (Alderman & Lacy, 1984).

There is a strong effect of temperature on conidial production by *P. personata* (Fig. 7). The temperature response for this process is quite different from that for infection, where there was little effect between 15 and 26°C (Butler *et al.*, 1994). We worked with a susceptible genotype (TMV 2), but the effect of temperature on conidial production can vary among genotypes with different levels of host resistance to *P. personata* (Shew *et al.*, 1988). We did not explore the possibility that the effect of temperature on conidial production could be dependent on other environmental conditions. This has been observed in the case of a number of other pathogens (Cohen & Rotem, 1987).

In experiments with alternate high and low RH, there was a possibility that the change in RH could have induced hygroscopic release of conidia. This has been observed with *Cercospora beticola* (Meredith, 1967) and *B. maydis* (Hyre, 1973). In order to determine whether a similar phenomenon was occurring in the case of *P. personata*, we exposed a microscope slide, coated with a mixture of petroleum jelly and wax, in each controlled-humidity chamber during different night/day humidity experiments. The number

of conidia collected on each slide over a period of 24 h never exceeded 12, and was often zero. This evidence suggests that large numbers of conidia were not released by changes in RH during this series of experiments.

Although the majority of conidia were produced with high RH in the dark, the RH during the previous day had a strong effect on production during the night (Figs 3b and 4b). We are not aware of similar reports for other pathogens which distinguish between spore production during day and night. Reports of alternate wet-dry or high-low RH regimes have examined overall spore production, and indicate a reduction proportional to the length of the dry or low RH period (Alderman & Lacy, 1984; Alderman & Beute, 1987; Harrison & Lowe, 1989). It has usually been assumed that spore production continues when the RH exceeds the threshold (or during dew periods), and ceases when the RH falls below the threshold (Nelson & Tung, 1973; Alderman & Lacy, 1984; Harrison & Lowe, 1989). Our findings indicate that the daytime (low) humidity value (Figs 3b and 4b) and the length of the high humidity period (Fig. 5) produce effects of similar magnitude.

The effects of several weather variables on conidial production have been considered here, and other variables, such as wind and rain, will affect spore dispersal (Smith & Crosby, 1973). Attempts to relate airborne spore catches to weather variables should consider both conidial production and dispersal. At times of favourable RH (usually at night), spore production by other pathogens of a similar nature to *P. personata* may also be influenced by the RH during the previous day. Further work is needed to verify this hypothesis, but such an effect could help to explain inconsistencies between field observations and results obtained from controlled-environment studies (e.g. Wallin & Loonan, 1977).

The studies on conidial production reported here have attempted to separate the effects of temperature from those of relative humidity. However, under natural conditions these two variables are interdependent; a fall in RH is invariably accompanied by a rise in temperature. Nevertheless, these results indicate that conidial production occurs predominantly at night when RH values tend to be higher and temperatures vary less than during the daytime. Overall, the results suggest that at least three important factors affect conidial production: (1) night-time temperature; (2) duration of RH > 95%; and (3) previous daytime RH. Further studies should aim to confirm the effects of these factors in the field.

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