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Factors influencing the production of basidiocarps and the deposition and germination of basidiospores of *Crinipellis perniciosa*, the causal fungus of witches' broom on cocoa (*Theobroma cacao*)

H. M. ROCHA* *and* B. E. J. WHEELER
Department of Pure and Applied Biology, Imperial College, Silwood Park, Ascot, Berkshire SL5 7PY, UK

The production of basidiocarps by *Crinipellis perniciosa* on detached, dead witches' brooms from cocoa was assessed in relation to temperature, light, cocoa clone, age of broom and type of tissue, in cabinets with a daily cycle of 8 h wet and 16 h dry. More basidiocarps formed and matured at 20–25°C than at 25–30°C. In the latter regime the pilei were smaller and white, instead of the usual crimson colour, and the stipes were longer. No basidiocarps formed at 30–35°C. At 20–25°C, more basidiocarps formed and matured with light at 100 μE m^{-2} s^{-1} during the wet period than at 10 μE m^{-2} s^{-1}. Only one basidiocarp and five primordia developed on 20 brooms kept in the dark. Brooms from 10 cocoa clones at Pichilingue, Ecuador, differed in basidiocarp productivity, most basidiocarps forming on brooms from Scavina and least on ICS clones. The numbers of basidiocarps produced on brooms aged 1, 2, 3 or 4 months when detached from cocoa trees were similar but time to initiation of the first primordium differed considerably. More basidiocarps formed at nodes than internodes.

The discharge of basidiospores was optimal at 20–25°C and 80% RH; germination was optimal in water agar films. Neither process was dependent on light.

INTRODUCTION

Infection of stem meristems on cocoa (*Theobroma cacao* L.) by the basidiomycete fungus *Crinipellis perniciosa* (Stahel) Singer (Holliday, 1970; Pegler, 1978) often results in the formation of swollen shoot systems, with shortened internodes, commonly termed witches' brooms. These green brooms subsequently die and later, in suitable environments, basidiocarps of the fungus form on them (Baker & Holliday, 1957; Solórzano, 1977; Aranzazu, 1981; Evans & Solórzano, 1982). Basidiocarps never form on green brooms which contain a mycelium of relatively thick (5–20 μm wide), intercellular hyphae considered to be the monokaryotic phase of the fungus. This has not been grown on agar media. The mycelium in the dead broom is composed of thinner hyphae (1·5–3·0 μm wide) with clamp connections and is thought to be dikaryotic (Pegus, 1972; Evans, 1980; Calle *et al.*, 1982). It can be isolated readily and grows well on many simple agar media but most attempts to induce basidiocarps from such mycelia in culture have failed (Delgado, 1974; Suarez-Capello, 1977). Stahel (1919) apparently obtained basidiocarps on mycelial mats hung outside and, more recently, Purdy *et al.* (1983) obtained basidiocarps on mycelial mats hung in chambers with an intermittent spray for 2 weeks. Also, Merchan (1979) reported that basidiocarps formed on cocoa stems about 4 months after they were sterilized and inoculated with mycelial plugs.

Cocoa can only be infected using basidiospores as inoculum, so infections in the field depend initially on the formation of basidiocarps on dead brooms. To date, this has also been true of research programmes such as testing for disease resistance which involve inoculations of cocoa plants. Yet the factors

*Present address: Centro Nacional de Recursos Geneticos, Parque Rural, SAIN, Cx Postal 10.2372, Brasilia — DF, Brazil.

which influence the formation of basidiocarps are little understood. The limited field data indicate that frequent but intermittent light showers favour basidiocarp formation (Baker & Holliday, 1957). This type of regime, involving alternate wetting and drying, was used by Suarez-Capello (1977) to induce basidiocarps on detached, dead brooms in cabinets and was developed further by Rocha & Wheeler (1982). They tested four daily regimes of successive wet and dry periods: (A) 1 h wet, 23 h dry; (B) 8 h wet, 16 h dry; (C) 16 h wet, 8 h dry; (D) 23 h wet, 1 h dry. Regime B proved most favourable for basidiocarps, which formed on 92% of the brooms in successive flushes over the 40 weeks of the experiment. Basidiocarp production appeared to be linked with changes in the water content of the brooms from 50% in the wet period to 15% in the dry period.

The effects of some environmental factors on the discharge and germination of basidiospores of *C. perniciosa* have been studied by several investigators but the data are relatively limited. The first report was made by Stahel (1919) who found that basidiospores were liberated mainly at night; he suggested that changes of temperature were responsible. Later, Baker & Crowdy (1943) found that the main requirements for basidiospore discharge from fully expanded, turgid pilei were temperatures between 12 and 30°C with relative humidities between 70 and 90%. Generally, in Trinidad, when the relative humidity was less than 70% little deposition occurred. In experiments with optimum humidity, the length of the deposition period ranged from about 4 h at 27°C to a maximum of 24 h at *c.* 16°C. Recently, Bastos (1982) also reported that the optimum range of temperature for basidiospore liberation is 15–25°C.

The research of Baker & Crowdy (1943) indicated that basidiospore germination may start within 2 h of deposition in a saturated atmosphere and be almost complete 4 h later. No germination occurred below 12°C or above 30°C, the same limits as they found for basidiospore deposition.

The studies reported in this paper examined other factors which were thought likely to influence basidiocarp production — temperature, light, cocoa clone, age of broom and sites of fungal activity within brooms. They examined also the effects of temperature, humidity and light on the deposition of basidiospores and of temperature and light on basidiospore germination. The overall aims were to determine the best conditions for a regular supply of basidiocarps on brooms and for obtaining deposition and germination of basidiospores, to aid research programmes and to provide information that might lead to a better understanding of the behaviour of the fungus in the field.

MATERIALS AND METHODS

General regime for producing basidiocarps

Dry fan brooms were imported under licence (PHF 29/119, Ministry of Agriculture, Fisheries and Food, England and Wales) from Castanhal near Belém, Pará, East Brazil and Ouro Preto, Rondonia, West Brazil and from Pichilingue in Ecuador. The brooms were kept in cardboard boxes in the laboratory (15–20°C) until required for experiments. Brooms were then hung individually within a cabinet which consisted of polyethylene sheeting fixed, except on one side to allow access, to a steel frame, 1·0 m high, 0·7 m wide, 0·7 m deep (Suarez-Capello, 1977) and were subjected to a daily regime of 8 h wet and 16 h dry. Wet periods were obtained with a Defensor 505 humidifier placed in each cabinet and linked with a time switch. During dry periods the humidifiers were switched off and the cabinets were opened to allow free circulation of air and drying of the brooms (Rocha, 1983).

Factors influencing the production of basidiocarps

Temperature

Electric tubular heaters (Humex Ltd) and a thermostat were installed in each of three cabinets to obtain three temperature regimes: 20–25°C; 25–30°C; 30–35°C. In the 20–25°C regime, temperatures tended to be at the upper limit of the range during the wet periods and at the lower end during the dry periods when the cabinet was opened. In the other two regimes, temperatures tended to be lowest during the wet period, possibly as a result of cooling by the water spray from the humidifier. These fluctuations were monitored with a thermohygrograph (Casella Ltd). All cabinets were housed in a room at 20°C, illuminated (60 $\mu E\ m^{-2}\ s^{-1}$) during the dry period.

Ten brooms from Castanhal were hung in each cabinet and the numbers of primordia and mature basidiocarps of *C. perniciosa* were counted weekly for 6 months. During this period, 20 mature basidiocarps were taken from each temperature regime, the diameter of their pilei and length of stipes were measured and also the dimensions of 100 basidiospores.

Light

Three cabinets were prepared, each at 20–25°C, in a room at 20°C illuminated at 60 μE m^{-2} s^{-1} for 8 h corresponding to the wet period. One cabinet was left unmodified; measurements with a Solartron 7040 light meter inside this cabinet indicated a light intensity of 10 μE m^{-2} s^{-1}. Two white fluorescent tubes (Atlas warm white, 65–80 W) were placed on each of two sides of a second cabinet and these gave a light intensity in the cabinet of 100 μE m^{-2} s^{-1} during the wet period. All measurements of light intensity were made at positions to be occupied by brooms. The third cabinet was enclosed in black polyethylene sheeting. This gave complete darkness during the wet period and, apart from a short period to inspect brooms, this cabinet remained in darkness because the light in the room was switched off during the dry period.

Twenty brooms from Castanhal were hung in each cabinet and the numbers of primordia and mature basidiocarps formed on them were counted weekly over 6 months.

Cocoa clones

Ten brooms of similar size were collected in August 1981 from several trees of the following clones in the cocoa germplasm collection at Pichilingue, Ecuador: UF 168, UF 677, Scavina 6, Scavina 12, ICS 1, ICS 39, ICS 95, IMC 67, EET 400 and Catongo. These brooms were hung individually in cabinets at 20–25°C, within a room illuminated at 60 μE m^{-2} s^{-1} on 7 November 1981. The numbers of primordia and mature basidiocarps which developed on each broom were counted weekly for 7 months.

Age of broom

During 1981 groups of 10 brooms were labelled from their initiation within a commercial planting of cocoa hybrids at Ouro Preto on four occasions: 27 March, 28 April, 28 May and 30 June. All brooms were harvested on 30 July and were sent to Silwood Park. They were allowed to dry and were then hung in cabinets at 20–25°C within an illuminated room (60 μE m^{-2} s^{-1}) on 2 September. The time taken for the first basidiocarp primordium to appear was noted and the total numbers of primordia and mature basidiocarps produced were counted over 9 months.

Sites within the broom

In previous experiments most basidiocarps appeared to form at the nodes of the hypertrophied shoots within the dead broom, so this was examined. Since nodes are areas from which leaves or side shoots arise and are then broken off, resulting in wounds, the effect of wounding brooms was incorporated in this experiment. Wounding consisted of removing a V-shaped wedge of tissue *c.* 1 cm long and 3–5 mm deep with a sharp scalpel. There were three treatments: none, one wound at a node, two wounds between nodes (internodes). Seven brooms of about equal size, from Pichilingue, Ecuador, were used in each treatment and they were kept in cabinets at 20–25°C within an illuminated room (60 μE m^{-2} s^{-1}). The numbers of mature basidiocarps were counted over 9 months.

Effects of temperature, relative humidity and light on basidiospore discharge

Basidiospore discharge was examined at 10, 15, 20, 25 and 30°C, at relative humidities of 80, 85, 90, 95 and 100%, both in the light (10 μE m^{-2} s^{-1}) and in the dark. Thus, there were a total of 50 treatment-combinations. Because many basidiocarps were required for all these treatments it was not possible to combine them in one factorial experiment. Instead, there were 10 consecutive experiments, one for each combination of temperature and light treatment. Each was set up as follows.

Fifteen newly formed basidiocarps of an isolate of *C. perniciosa* from Castanhal were selected with pilei of similar diameter (*c.* 1 cm). The pilei were excised and were fixed on the underside of lids of polystyrene boxes (10 × 10 cm) with Vaseline so that the gills faced the

bottom of the box. The base of each box had 25 compartments (2 × 2 cm). Two ml of distilled water were placed in each of three compartments and three pilei were positioned correspondingly on the lid so that basidiospores could discharge into each of these compartments. The lid was placed over the base leaving a space to permit free circulation of air. Five boxes were prepared in this way. Each of these was then placed in a larger (17·5 × 11·5 cm), covered plastic box containing 100 ml of a solution of glycerine in distilled water (51, 44, 32·5 or 12·5 ml glycerine per 100 ml solution) or 100 ml distilled water to give relative humidities of 80, 85, 90, 95 or 100% (Booth, 1971). These boxes were then kept in an incubator at one of the designated temperatures, either illuminated or not. Basidiospores were allowed to deposit for 4 h; a drop of Tween 20 was then added to each suspension and the number of basidiospores was estimated from haemocytometer counts.

Effects of temperature and light on basidiospore germination

Basidiospores were allowed to deposit from basidiocarps of an isolate of *C. perniciosa* from Castanhal on microscope slides covered with a thin layer of water agar (1 g agar per 100 ml). Three pilei were suspended over each slide to give three separate spore prints. Each slide was then placed in a covered plastic box on wet blotting paper and incubated at one of the following temperatures: 10, 15, 20, 25 and 30°C, with or without light (10 μE m^{-2} s^{-1}). After 3 h a drop of cotton blue in lactophenol was placed on each spore print. Germination was assessed from counts of basidiospores within five microscope fields (× 400) in each spore print giving over 500 basidiospores in each treatment. The lengths of 20 germ-tubes in each treatment were also measured.

Analysis of results

Rocha & Wheeler (1982) showed that the numbers of basidiocarps produced on a small collection of brooms did not fit a normal distribution, therefore populations of basidiocarps were compared using the non-parametric Mann-Whitney *U* test (Siegel, 1956). Measurements of basidiocarps and basidiospores produced in two different treatments were compared by *t*-tests and the proportions of basidiocarps maturing in different regimes were compared by chi-squared tests. The numbers of basidiospores deposited in different regimes were compared using analysis of variance and regression analysis (Steel & Torrie, 1960).

RESULTS

Factors influencing the production of basidiocarps

Temperature

No basidiocarps were produced at 30–35°C. Comparisons of basidiocarp populations in the other two regimes indicated that the numbers of primordia and mature basidiocarps produced at 20–25°C were significantly greater than on brooms kept at 25–30°C at $P = 0{\cdot}0136$ and $P = 0{\cdot}0008$ respectively (Table 1). The percentage of basidiocarps which matured at 20–25°C was also significantly greater ($\chi^2 = 8{\cdot}67$, $P < 0{\cdot}01$) than at 25–30°C. At 25–30°C the stipes of the basidiocarps were significantly longer ($P < 0{\cdot}001$) and the caps were smaller ($P < 0{\cdot}05$) than those of basidiocarps produced at 20–25°C (Table 2) but, most strikingly, the pilei were white instead of the usual crimson colour.

Light

The different light regimes affected basidiocarp production markedly. Significantly more primordia ($P = 0{\cdot}026$) and mature basidiocarps ($P = 0{\cdot}0003$) were produced on brooms illuminated at 100 μE m^{-2} s^{-1} than on those at 10 μE m^{-2} s^{-1} (Table 3), although the numbers of brooms producing basidiocarps were similar (15 and 14 brooms respectively). Also, the percentage of basidiocarps which matured at 100 μE m^{-2} s^{-1} was significantly greater ($\chi^2 = 6{\cdot}03$, $P < 0{\cdot}05$) than at 10 μE m^{-2} s^{-1}. Only five primordia and one mature basidiocarp formed during the 6 months on brooms kept in the dark. However, on these brooms there were many mycelial aggregates like those which precede recognizable primordia, suggesting that light promotes the development of primordia and possibly their further growth.

Cocoa clones

There were some differences in the production

Table 1. Effect of temperature on the production of basidiocarps of *Crinipellis perniciosa*

Temperature regime (°C)[a]	Basidiocarp development	Numbers produced in successive 4-week periods[b]				Totals
		3	4	5	6	
20–25	Primordia	3	42	29	22	96
	Mature	1	33	28	13	75
25–30	Primordia	10	19	13	1	43
	Mature	0	13	9	1	23

[a]Both regimes with cycle of 8 h wet, 16 h dry per day.
[b]Each figure is the total number produced on 10 brooms over 4 weeks; no basidiocarps were produced in the first two 4-week periods.

Table 2. Effect of temperature on the morphology of basidiocarps and basidiospores of *Crinipellis perniciosa*

Character	Temperature regime (°C)	
	20–25	25–30
Pileus colour	Crimson	White
Pileus diameter (mm), range	4–22	6–12
mean ± SE	10·6 ± 1·07	8·0 ± 0·28
Stipe length (mm), range	6–11	7–12
mean ± SE	7·7 ± 0·34	10·8 ± 0·31
Basidiospores, length (μm)	11·6	11·9
breadth (μm)	6·4	6·5
length/breadth	1·8	1·8

Table 3. Effect of light intensity on the production of basidiocarps by *Crinipellis perniciosa*

Light intensity (μE m^{-2} s^{-1})[a]	Basidiocarp development	Numbers produced in successive 4-week periods[b]				Totals
		3	4	5	6	
100	Primordia	5	48	94	18	165
	Mature	3	31	63	16	113
10	Primordia	0	21	22	11	54
	Mature	—	8	16	3	27
0	Primordia	0	0	5	0	5
(darkness)	Mature	—	—	1	—	1

[a]All regimes with a cycle of 8 h wet, 16 h dry per day; illumination, where given, during the wet period.
[b]Each figure is the total number produced on 20 brooms over 4 weeks; no basidiocarps were produced in the first two 4-week periods.

of basidiocarps on brooms from the different cocoa clones, the mean numbers of mature basidiocarps ranging from 21 on producing brooms of Scavina 6 to 5·3 on those of ICS 1 (Table 4). Comparisons of the populations of basidiocarps produced on any two clones, using the Mann-Whitney U test, showed that the numbers of mature basidiocarps on brooms of Scavina 6 were significantly greater ($P < 0{\cdot}02$) than on brooms from all other clones except Scavina 12. Zero values for four brooms of UF 168 limited the analysis but a comparison of this clone with Scavina 6, excluding these zero values, indicated that significantly ($P < 0{\cdot}01$) more mature basidiocarps were produced on brooms from Scavina 6.

Age of broom

The numbers of primordia and mature basidiocarps produced on brooms of different age when detached from the cocoa tree did not differ significantly (Table 5).

However, the time taken for the first primordium to appear varied considerably. As a green broom takes approximately 1–2 months to become dry, the minimum period from this point to start basidiocarp production ('dormant'

Table 4. Basidiocarps of *Crinipellis perniciosa* produced on brooms from different cocoa clones at Pichilingue, Ecuador, over 7 months

Clone	No. brooms producing basidiocarps (out of 10)	Mean No. per producing broom	
		Primordia	Mature
Scavina 6	10	26·4	21·0
UF 168	6	24·6	17·3
Scavina 12	10	17·9	12·4
IMC 67	10	13·4	10·0
Catongo	10	13·2	8·6
EET 400	10	12·1	8·0
UF 677	8	9·7	7·6
ICS 39	10	10·2	7·2
ICS 95	8	9·6	6·0
ICS 1	9	7·2	5·3

Table 5. Basidiocarps of *Crinipellis perniciosa* produced on brooms of different age collected at Ouro Preto, Rondonia, Brazil in 1981

	Brooms formed on			
	27 Mar.	28 Apr.	28 May	30 June
Age of brooms (months)				
At harvesting	4	3	2	1
When placed in cabinet	5	4	3	2
Days from harvesting to first primordium				
Mean	88	105	109	136
Range	59–158	59–191	89–188	93–196
Mean No. basidiocarps per producing broom[a]				
Primordia	31	25	29	23
Mature	24	22	21	18

[a]Each figure is based on the total production on 10 brooms over 9 months.

period) appears to be at least 3 months. This is indicated by the data for brooms formed by 30 June.

Sites within the broom

Most basidiocarps were produced at nodes, the differences in numbers at nodes and internodes being very highly significant ($P < 0{\cdot}001$, Table 6). The total number of basidiocarps produced in each of the three treatments (110, no wounding and 147 and 127 for wounding at nodes and internodes respectively) did not differ significantly from a 1:1:1 ratio ($\chi^2 = 5{\cdot}36$, $P > 0{\cdot}05$). Therefore it appears that wounding did not affect their production.

Effects of temperature, relative humidity and light on basidiospore discharge

The mean numbers of basidiospores discharged in the 10 experiments are shown in Table 7. Analyses of variance indicated that, overall, temperature and humidity affected basidiospore discharge significantly ($P < 0{\cdot}001$) but light/dark did not ($P > 0{\cdot}05$). There was also a very highly significant ($P < 0{\cdot}001$) temperature × humidity interaction.

Table 6. Basidiocarps of *Crinipellis perniciosa* produced on brooms from Pichilingue at nodes and internodes with or without wounding

Treatment[a]	Site of basidiocarps	Total number produced over 9 months[b]	χ^2 [c]	P
None	Node	102	80·3	< 0·001
	Internode	8		
Wounds at node	Node	129	83·3	< 0·001
	Internode	18		
Wounds at internode	Node	116	86·8	< 0·001
	Internode	11		

[a]All brooms were kept at 20–25°C with cycle of 8 h wet and 16 h dry.
[b]Total for seven brooms.
[c]For departure from a 1:1 ratio.

Table 7. Effects of temperature, relative humidity and light on the discharge of basidiospores of *Crinipellis perniciosa*

Temperature (°C)	Light (L) or dark (D)	Thousands of basidiospores deposited[a] in 4 h at RH of					Mean
		80%	85%	90%	95%	100%	
10	L	13	12	15	7	17	12·8
	D	11	8	13	4	12	9·6
15	L	156	263	217	191	225	210·4
	D	167	281	240	171	184	208·6
20	L	358	476	431	348	371	396·8
	D	332	424	437	343	368	380·8
25	L	584	575	445	376	304	456·8
	D	483	480	388	365	300	403·2
30	L	56	63	113	11	167	82·0
	D	48	71	93	39	100	70·2
Mean		220·8	265·3	239·2	185·5	204·8	

[a]Each figure is the mean of three replicates.

The effects of temperature and humidity were examined further by regression analysis with basidiospore number as dependent variable. The regression of basidiospore number on temperature was best represented by the equation $y = 140\ x - 21{\cdot}97\ x^2 - 124{\cdot}88$, this relationship being very highly significant ($P < 0{\cdot}001$). Separate regressions of basidiospore number on temperature at each of the five relative humidities were basically similar. All were described best by quadratic equations, the regressions being very highly significant ($P < 0{\cdot}001$). The results generally indicated an optimum temperature for basidiospore discharge near 25°C.

The overall linear regression of basidiospore number on humidity was not significant, nor were most of the separate regressions on humidity at each temperature for basidiocarps kept in the light or dark. However, at 25°C, these linear regressions were highly significant ($P < 0{\cdot}001$). The regression coefficients were negative but that for basidiocarps in the light ($-17{\cdot}97$) was not significantly different from that for basidiocarps in the dark ($-12{\cdot}0$).

Effects of temperature and light on basidiospore germination

No basidiospores germinated at 10°C. Germination was poor at 15°C and optimal at 25°C with corresponding effects on germ-tube growth (Table 8). Light did not affect germination or germ-tube growth.

DISCUSSION

There have been few observations on the effects of light and temperature on fruiting of *C. perniciosa* (Reyes & Reyes, 1976) but the present studies indicate that these factors are important, as they are with other basidiomycetes (Manachere, 1980). When the water requirements were satisfied, temperatures between 20 and 25°C and a light intensity of 100 $\mu E\ m^{-2}\ s^{-1}$ were particularly favourable for basidiocarp formation. Temperatures over 30°C were inhibitory but such temperatures are not common during wet periods in regions of South America where cocoa is grown (Wood, 1975). The change in the morphology of the fungus at 25–30°C is more interesting, especially the lack of red pigment, because such features could be taken as sufficient for designating such a form as a different variety of *C. perniciosa*. Few such variants have been found in nature. Pegler (1979) recognizes three: var. *perniciosa* and var. *ecuadoriensis*, both with red caps, and var. *citriniceps* with a citron yellow cap. The last named variety was recorded by Evans (1978) on a broom at Pichilingue, Ecuador. Again, it is unlikely that basidiocarps in the field would experience temperatures in this range because they develop mainly during wet periods when, at least during the night, temperatures are lower.

It is of particular interest that brooms from different cocoa clones at Pichilingue supported different numbers of basidiocarps because this suggests that within local areas the production

Table 8. Effects of temperature and light on the germination of basidiospores of *Crinipellis perniciosa*

°C	Light (L) or dark (D)	% germination[a] ± SE	Germ tube length[b] (μm) ± SE
15	L	17·3 ± 1·1	4·8 ± 0·4
	D	16·1 ± 1·2	5·0 ± 0·4
20	L	74·1 ± 2·9	29·6 ± 2·5
	D	71·7 ± 2·4	25·1 ± 3·2
25	L	85·7 ± 1·7	79·3 ± 3·5
	D	90·0 ± 0·2	80·3 ± 3·4
30	L	57·6 ± 2·1	67·9 ± 3·0
	D	58·7 ± 1·4	67·6 ± 3·4

[a]Mean % germination (as angular transforms) after 3 h; based on 15 replicate counts giving a total of > 500 basidiospores.
[b]Mean of 20 replicates.

of inoculum might be influenced considerably by the type of cocoa grown. Most basidiocarps were produced on brooms from Scavina 6 which in other experiments (Wheeler & Mepsted, 1982) proved susceptible to isolates of *C. perniciosa* from Ecuador but relatively resistant to isolates from Brazil. Fewer basidiocarps developed on ICS clones 1 and 95. Apart from size of broom, which was kept fairly uniform, it could be argued that other factors influencing basidiocarp production were not controlled in this experiment. Samples were taken at random from the trees and could thus have included brooms of different age. It is possible that the range of basidiocarp productivity from Scavina 6 to ICS 1 relates to increasing quantities of old, unproductive brooms in samples, though this seems unlikely. Ideally, the productivity of brooms on the different cocoa clones needs to be studied on site or with brooms of known age to check the present experimental results. Disease incidence at Pichilingue is high and any feature of the cocoa which tends to reduce inoculum is potentially useful, especially since ICS clones generally yield well.

The experiment on brooms of different age suggests that a minimum period of 4 months is required after brooms form before basidiocarps can develop on them. Aranzazu (1981) obtained a similar result in field conditions in Colombia. Such data are useful in planning sanitation programmes. The relative abundance of basidiocarps at nodes probably indicates the preference of the fungus for meristematic sites within the developing shoots. It also indicates that a practical way of standardizing broom material for comparative assessments of their productivity would be to take lengths of broom with equal numbers of nodes.

The processes of basidiospore discharge, germination and germ-tube growth were affected considerably by temperature. Basidiospores were liberated between 10 and 30°C but germination occurred only between 15 and 30°C. These results agree with most of the field observations and experimental data reported in the literature although Baker & Crowdy (1943) found that at 12°C no basidiospores were discharged from basidiocarps.

The effects of humidity on basidiospore discharge are less easy to interpret because in this series of experiments temperature effects, which were considerable, were confounded with days on which each experiment was done and thus with the particular crop of basidiocarps. Generally, over the range 10–30°C, humidity had little effect on basidiospore discharge. Although as a factor in the overall analysis of variance humidity was significant, the linear regression of basidiospore number on humidity was not significant. However, there was a significant temperature × humidity interaction which related to the two experiments at 25°C, one in the light and one in the dark. In these experiments there were negative relationships between numbers of basidiospores discharged and relative humidities ranging from 80 to 100%. These particular results suggest that at 25°C, which is near optimal for basidiospore discharge, humidity affected the process. They agree with the suggestion of Baker & Crowdy (1943), based on field observations, that a relative humidity around 85% might be most favourable for basidiospore discharge.

The data otherwise suggest that temperature, humidity and light are unlikely to limit the production of inoculum and its germination since, during rainy periods, temperatures between 20 and 25°C and relative humidities between 80 and 90% are common in most areas in South America and the Caribbean where cocoa is grown.

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