THE ECOLOGY OF *CRINIPELLIS PERNICIOSA* (Stahel) Singer IN WITCHES' BROOMS ON COCOA (*THEOBROMA CACAO* L.)

by

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ABSTRACT

The production of basidiocarps of *Crinipellis perniciosa* on dead witches' brooms was assessed in relation to different regimes of wetting and drying, temperature, light, the type of cocoa, age of broom and type of tissue. Most basidiocarps formed when brooms were subjected to successive periods of 8 h wet/16 h dry at 20-25° C and were illuminated at 100 $\mu E/m^2/s$. Basidiocarps formed most prolifically on Scavina clones 6 and 12 from Ecuador and at nodes rather than internodes.

The discharge of basidiospores was optimal at 20-25° and at relative humidities c. 80%; germination was optimal at 25° in water agar films and was not dependent on light. Infection, symptom development and broom formation by different pathotypes of *C. perniciosa* were examined on several types of cocoa. Scavina 6 and Scavina 12 showed a resistant reaction when inoculated with the Brazilian pathotype while IMC 67 and Catongo were susceptible. All types were susceptible to the Ecuadorian pathotype. Symptom development was most severe at 25° and 30° C.

A method was developed for determining fungal biomass in dead brooms based on an assay of the glucosamine content of ground broom tissue. Fungal biomass increased markedly when green brooms died and was greatest in the cortex of the node, where most basidiocarps formed. Also, fungal biomass was higher in brooms subjected to a regime of 16 h wet/8 h dry than in other regimes and in infected plants kept at 30° compared with lower temperatures.

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CONTENTS

GENERAL INTRODUCTION

Witches' broom caused by the basidiomycete fungus *Crinipellis perniciosa* (Stahel) Singer is the major factor limiting cocoa production in South America and the Caribbean, except in the Bahia region in Brazil. The absence of witches' broom in Bahia, where approximately 500 000 hectares of cultivated cocoa are grown is due to a natural barrier of a dry region some 2000 km across which separates Bahia from Amazonia where the disease is endemic in wild cocoa and epidemic in the cultivated crop. The chances of its introduction into Bahia by man unintentionally carrying infected material have increased in recent years due to the expansion of cocoa production in Amazonia and, particularly because communication between the two areas has improved.

The disease also poses a threat to those cocoa growing areas in Central America, West Africa and Asia where, so far, it has not been reported. Quarentine measures have been taken to avoid its introduction into disease-free areas not only by the Brazilian authorities in respect of Bahia but also by the authorities of other cocoa producing countries.

Despite its devastating effect, information about witches' broom especially the ecology of the fungus and the epidemiology of the disease, is limited compared with other important diseases. Some valuable basic research has been done in Trinidad during the 1940's and 1950's and more recently in Ecuador and Brazil but for various reasons this research has often lacked continuity.

This investigation attempts particularly to extend studies on the ecology of the fungus in dead brooms.

The influence of wetting and drying, temperature, light, cocoa cultivars and age of brooms, on the production of basidiocarps was studied and this led to further experiments on factors influencing the discharge of basidiospores,

their requirements for infection and the development of the fungus in broom tissue.

REVIEW OF LITERATURE

1. The fungus

The casual fungus of witches' broom was first described by Stahel (1915) as *Marasmius perniciosus*. This is a member of the sub-class Homobasidiomycetidae, series Hymenomycetes, order Agaricales and family Agaricaceae (Alexopoulos, 1962). Later, in a revision of the Agaricales, Singer (1942) transferred it to the sub-section Iopodinae of the genus *Crinipellis* as *C. perniciosa*, by which name it is currently known.

The fungus is endemic to the forests of the Amazon basin attacking cocoa and related species of *Theobroma* and *Herrania*, and is a highly destructive pathogen in cocoa plantations in the northern regions of South America and in the Caribbean Islands (Baker & Holliday, 1957).

2. The disease

(a) Infection: According to Stahel (1919) infection takes place via stomata of new shoots, young leaves and pods. There are indications that penetration of the cuticule can occur on seedlings not more than 4 days old, provided sufficient embryonic tissue is present (Holliday, 1955). Generally a germ tube from a basidiospore becomes positioned over the junction between cells and its tip becomes swollen before penetration takes place, (Suarez-Capello, 1977). More recent, experimental evidence indicates that stem, leaf petioles, and pulvini are also vulnerable to infection, as well as wounds in woody and non-woody cocoa tissues (Evans *et al.*, 1977; Cronshaw & Evans, 1978). There are no precise data relating infection with climatic factors such as temperature.

(b) Symptom development: The classic symptoms of witches' broom were first properly described by Went (1904). They consist mainly of abnormal

proliferation of buds resulting in flushes of swollen shoots with excessive branching and shortened internodes to which the term 'broom' is applied. These brooms may develop at the ends of shoots ('terminal brooms') or occur laterally ('lateral brooms'). Occasionally infections are not associated with buds. In these instances, symptoms appear as a roughening or swelling of the twig, often associated with internal discolouration, and this affected area later develops into a canker (Thorold, 1975). The flower cushion can also be infected. This results in the development of swollen infected shoots and abnormal flowers collectively known as a 'star-bloom'. Infected flowers are abnormally large, with thickened stalks and they stand out from the cushion instead of hanging limply like healthy flowers. They are persistent while normal flowers absciss after 3 days if unfertilized (Baker & Crowdy, 1943). Young pods can be infected either systemically by hyphae growing from an infected cushion through the stalk or directly by germ tubes from germinating basidiospores. Systemic infection gives rise to abnormal, round or strawberry shaped fruits known as 'chirimoyas'. These are parthenocarpic, pollination not being required for their formation (Cronshaw & Evans, 1978). Infection by basidiospores through the stomata of pods (c. 1.0 cm long) gives a characteristic 'carrot-shaped' fruit. On older pods, 2-3 cm long, infection causes a one-sided distortion; the pods do not ripen normally and the beans rot (Thorold, 1975). Infection of larger pods results in a hardening of the tissues ('induration') but no hypertrophy.

The factors influencing the rate of symptom development, the diversity of symptoms and the severity of their expression have not been well established.

(c) Broom formation: The characteristic symptom picture or syndrome of witches' broom is the formation of 'green' brooms following infection of meristems by *C. perniciosa* and subsequently the death of these brooms and

the formation of the fungus' basidiocarps on them (Baker & Holliday, 1957). The transition between the 'green' period and the 'dead' period of the broom appears to mark the limits between the biotrophic and necrotrophic phases of the fungus but the nature of the events resulting in broom death or the initiation of the saprophytic phase of the fungus and the relationship between these two processes, if any, have not been determined. Recently, Wheeler & Mepsted (1982) have shown that the time taken for a green broom to die is partly influenced by the pathotype involved as well as the cocoa cultivar.

There are no references in the literature concerning the role of climatic factors such as temperature on the amount of swelling or the influence of cocoa cultivars and different isolates of *C. perniciosa* on the diversity and extent of brooming.

(d) Production of basidiocarps: After the death of the broom a period elapses before the start of basidiocarp production. The length of this period varies from a few weeks to several months (Baker & Crowdy, 1943). Basidiocarps are then produced when the dead brooms are subjected to successive wetting and drying during the rainy season (Baker & Holliday, 1957). The changes which take place within the dead broom that enable the production of basidiocarps are not known but presumably involve the further growth of the fungus particularly in the cortex from which the basidiocarp primordia arise.

Certainly the lack of sufficient knowledge about the factors influencing the production of basidiocarps has limited research on this disease especially the evaluation of cocoa germ plasm in the search for resistance. Also, the investigation of races or pathotypes of *C. perniciosa* depends on synchronizing basidiocarp production with the availability of young cocoa tissue (Wheeler & Mepsted, 1982). Some of the factors requiring investigation are:

the effects of successive wetting and drying the brooms particularly the intervals between them, temperature, light, cocoa cultivars, pathotypes of the fungus, age of the broom and competition with other micro-organisms.

(e) Basidiospore discharge and germination: According to Holliday (1970), basidiospore discharge is optimal at high humidities between 16° and 27° C. This statement is based largely on work by Baker & Crowdy (1943) who reported limits of 12° and 30° C and whose field observations suggested that relative humidities between 70% and 90% were most favourable for basidiospore discharge.

These workers also found that relative humidities below 90% were unfavourable for germination. The optimum temperature range for germination appears to be between 15° C and 25° C. (Bastos, 1982). The effect of light either on the discharge of basidiospores or on their germination has not been reported.

SECTION I

FACTORS INFLUENCING THE PRODUCTION OF BASIDIOCARPS ON DEAD BROOMS

Introduction

Infection of cocoa stem meristems by *C. perniciosa* results in the formation of swollen shoot systems, with shortened internodes and excessive branching, commonly termed 'green brooms' (Fig I.1). These brooms subsequently die and later, in suitable environments, basidiocarps of the fungus form on them. The period over which the brooms remain green is rather variable and depends on the vigour of the plant and the pathotype of the fungus (Wheeler & Mepsted, 1982). It can vary from 4 to 18 weeks but is usually between 5 and 12 weeks. After the brooms die there is a 'dormant' period before basidiocarps start to form, the length of which also varies from a minimum of 6 weeks to a maximum of 66 weeks with the average between 12 and 32 weeks (Baker & Holliday, 1957; Soloranzo, 1977; Aranzazu, 1981). Basidiocarp production is then favoured by successive wetting and drying during a rainy season and especially by frequent, intermittent light showers (Baker & Holliday, 1957). The brooms retain their ability to produce basidiocarps for 2 years or more though the numbers produced tend to decline as the broom ages (Evans, 1981). Basidiocarps never form on green brooms which contain a mycelium of characteristically thick (4-20 μm wide), intercellular hyphae considered to be the monokaryotic phase of the fungus (Pegus, 1972; Evans, 1980).

The mycelium in the dead broom is composed of much thinner hyphae (1.5-4 μm wide) with clamp-connexions and is thought to be dikaryotic. 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

Fig I.1: Field symptom of witches' broom, showing a 'green broom' and abnormal fruits in a mature cocoa plant.

FIGURE I.1.

failed (Delgado, 1974; Suarez-Capello, 1977). However, a recent paper by Purdy, Trese & Aragundi (1983) reports the production of basidiocarps on mycelial mats hung in chambers with an intermittent water spray. Also, Merchan (1979) reported that basidiocarps formed on sterilized cocoa stems about 4 months after they were inoculated with plugs of mycelium taken from cultures of *C. perniciosa*.

Cocoa can only be infected using basidiospores as inoculum, so infections in the field depend entirely on the formation of basidiocarps on dead brooms. To date, this has also been true of laboratory tests, for example those which attempt to screen cocoa selections for resistance to witches' broom. The factors which influence the formation of basidiocarps are little understood. The studies reported in this section examined some factors which were considered likely to be important. These were regimes of wetting and drying, temperature, light, cocoa cultivar and age of broom. An attempt was also made to determine the utilization of cellulose and lignin by the fungus in dead brooms. The overall aim was to determine the best conditions for a regular supply of basidiocarps on brooms to aid research programmes and to provide information that might lead to a better understanding of basidiocarp formation in the field.

Experimental

(a) General regimes for producing basidiocarps

Dry fan brooms were imported under licence (PHF 29/119, Ministry of Agriculture, Fisheries and Food, England and Wales), from three areas of Brazil, Castanhal, Manaus and Ouro Preto. The brooms were kept in cardboard boxes in the laboratory (20°-25°C.) until required.

Twenty brooms from each region, 10 large (> 20cm long) and 10 small (< 20cm long) were hung individually inside each of four cabinets (Fig I.2). Each cabinet consisted of polyethylene sheeting fixed to a steel frame (1.0m high, 0.7m wide, 0.7m deep), except on one side to allow access (Suarez-

Fig. I.2: Cabinets used for basidiocarp production on dead brooms.

FIGURE I.2.

Capello, 1977). The brooms in each cabinet were subjected to one of the following regimes during successive 24 h periods: (A) 1 h wet, 23 h dry; (B) 8 h wet, 16 h dry; (C) 16 h wet, 8 h dry and (D) 23 h wet, 1 h dry. Wet periods (100% relative humidity) were obtained with a Defensor 505 humidifier (Atkiengesell Schaft, Zurich) 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. All cabinets were housed in an illuminated room (60 $\mu E/m^2/s$) at 20°-25°C.

The numbers of primordia and mature basidiocarps produced were counted weekly for 15 weeks. After this period, 10 brooms were transferred from regimes A, C and D to regime B and basidiocarp production was recorded for a further 4 weeks. The production of basidiocarps on brooms in regime B was recorded for an additonal 24 weeks, ie. 39 weeks overall. Once mature basidiocarps were counted they were removed from the brooms to avoid counting them twice.

In some regimes, notably A and D, basidiocarps did not form at all on many brooms (Table I.1). Where they did form the numbers produced did not fit a normal distribution. Therefore, populations of basidiocarps were compared using the non-parametric Mann-Whitney U Test (Siegel, 1956). Such comparisons showed that, although in each regime more basidiocarps were produced on large brooms than on small brooms from the same source, the populations were not significantly different, nor did broom size affect the percentage of primordia reaching maturity (Table I.2).

The data for large and small brooms were thus bulked for comparisons involving brooms from the three sources within any one regime (Table I.3). Analysis of these data indicated differences that were influenced by the regime in which the brooms were kept. Thus in regime B, the numbers of primordia and of mature basidiocarps produced on brooms from both Castanhal

TABLE I.1

Number of brooms from three regions of Brazil on which basidiocarps of *C. perniciosa* formed in four different regimes. [a]

Region	Regime (h wet/dry)				Total	%
	A (1/23)	B (8/16)	C (16/8)	D (23/1)		
Ouro Preto	0	17	13	3	33	41.2
Castanhal	4	19	15	13	51	63.7
Manaus	2	19	17	4	42	52.5
Total	6	55	45	20		
%	10	92	75	33		

[a] Sample size for each source within each regime : 20 brooms

TABLE I.2

Production of basidiocarps of *C. perniciosa* on large and small brooms in four different regimes.

Stage of development	Size of brooms	Regime (h wet/dry) A (1/23)	B (8/16)	C (16/8)	D (23/1)	Total
Total primordia	Large[a]	6[b]	622	226	77	931
	Small	3	414	175	27	619
Mature basidiocarps	Large	0	388	161	47	596
	Small	0	265	141	18	424
% basidiocarps reaching maturity	Large	0	62.4	71.2	61.0	
	Small	0	64.0	80.6	66.7	

[a] Large Brooms > 20cm long; small < 20cm long

[b] Each figure is the total production on 30 brooms (10 from each of the regions, Castanhal, Manaus and Ouro Preto).

TABLE I.3

Production of basidiocarps of *C. perniciosa* on brooms from three regions of Brazil in four different regimes.

Stage of development	Region	Regime (h wet/dry) A (1/23)	B (8/16)	C (16/8)	D (23/1)	Total
Total primordia	Ouro Preto	0	102[a]	137	4	243
	Castanhal	6	564	108	72	750
	Manaus	3	370	156	28	557
	Total	9	1036	401	104	1550
Mature Basidiocarps	Ouro Preto	0	67	108	2	177
	Castanhal	0	330	73	36	439
	Manaus	0	256	121	27	404
	Total	0	653	302	65	1020
% basidiocarps	Ouro Preto	0	65.7	78.8	50.0	
reaching maturity	Castanhal	0	58.5	67.6	50.0	
	Manaus	0	69.2	77.6	96.0	

[a] Each figure is the Total production of 20 brooms (10 large and 10 small).

and Manaus were significantly greater ($p < 0.001$) than on brooms from Ouro Preto, but in regime C there were no significant differences in the production of basidiocarps on brooms from the three sources. Similarly, the proportion of basidiocarps reaching maturity was not associated consistently with the source of the brooms.

The regime in which the brooms were kept was the main factor influencing basidiocarp production. For both total primordia and mature basidiocarps, the productivity in terms of regimes was B> C > D> A. Paired comparisons (Table I.4) for brooms from the same source showed that, with four exceptions, the numbers of basidiocarps differed significantly in each regime. The four exceptions were: the numbers of primordia on brooms from Castanhal did not differ in regime C and D nor those on brooms from Manaus in regimes A and D; the numbers of mature basidiocarps on brooms from Ouro Preto did not differ in regime B and C nor in regime A and D. However, two of these comparisons involved zero values or extremely low numbers.

The percentage of primordia reaching maturity varied from 50 to 96, but there was some indication that on brooms from the same source relatively more reached maturity in regime C than in regime B (Table I.3).

The patterns of basidiocarp production obtained by accumulating the weekly counts of primordia from brooms of all three sources, emphasize further the differences between regimes (Fig I.3). In the driest regime (A) no new primordia were initiated after 4 weeks and in the wettest regime (D), the rate at which primordia were initiated slowed considerably after 3 weeks. In the other two regimes (B and C), the numbers of new primordia continued to rise throughout the experiment though to different extents. However, for these regimes cummulative totals partly mask the characteristic flushes of basidiocarps which are evident from successive weekly totals (Fig I.4 and I.5).

TABLE I.4

Probabilities that populations of *C. perniciosa* basidiocarps differ in four regimes[a]

Stage of development	Regime	Ouro Preto			Castanhal			Manaus		
		B	C	D	B	C	D	B	C	D
Total primordia	A	< 0.001	< 0.001	< 0.001	< 0.001	<0.001	<0.001	<0.001	<0.001	0.17
	B		0.07	< 0.001		0.006	<0.001		0.003	<0.001
	C			< 0.001			0.30			<0.001
Mature Basidiocarps	A	< 0.001	< 0.001	0.077	<0.001	<0.001	<0.001	<0.001	<0.001	0.02
	B		0.412	0.002		<0.001	<0.001		0.007	<0.001
	C			< 0.001			0.042			<0.001

[a] Regimes (h wet/dry): A(1/23), B(8/16), C(16/8), D(23/1).

Fig I.3: Production of basidiocarps of *C. perniciosa* on brooms kept in different regimes: A, 1 h wet, 23 h dry (△); B, 8 h wet, 16 h dry (○); C, 16 h wet, 8 h dry (●); D, 23 h h, 1 h dry (▲).

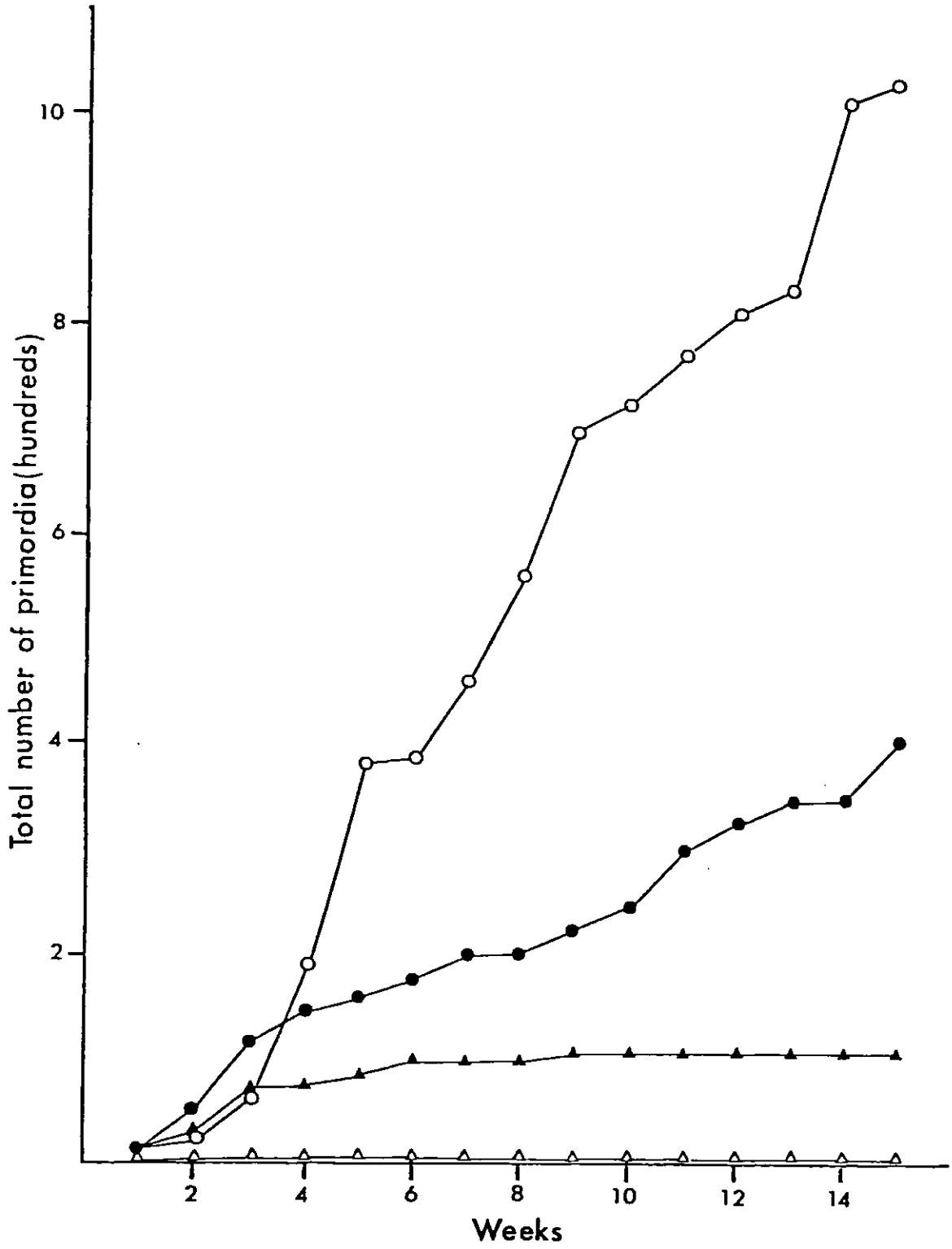

Total number of primordia(hundreds)
10
8
6
4
2
2
4
6
8
10
12
14
Weeks

FIGURE I.3

Fig I.4: Weekly production of basidiocarps of *C. perniciosa* by brooms in a regime of successive 8 h wet, 16 h dry periods. Brooms from (a), Castanhal; (b), Manaus and (c) Ouro Preto regions of Brazil.

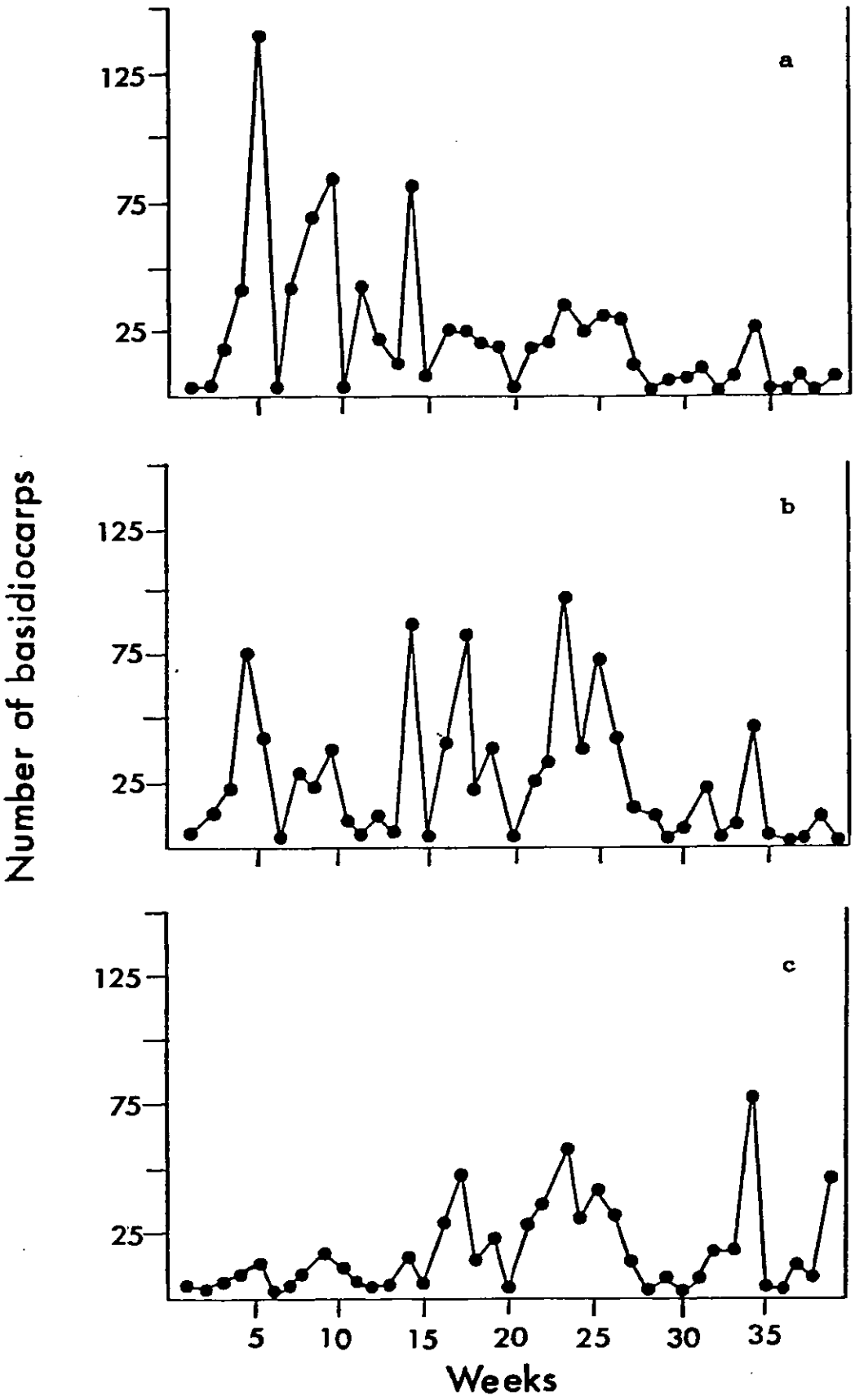


FIGURE I.4

Fig I.5: Flushes of basidiocarps on dead brooms kept at 20°-25° C with a regime of 8 h wet/16 h dry.

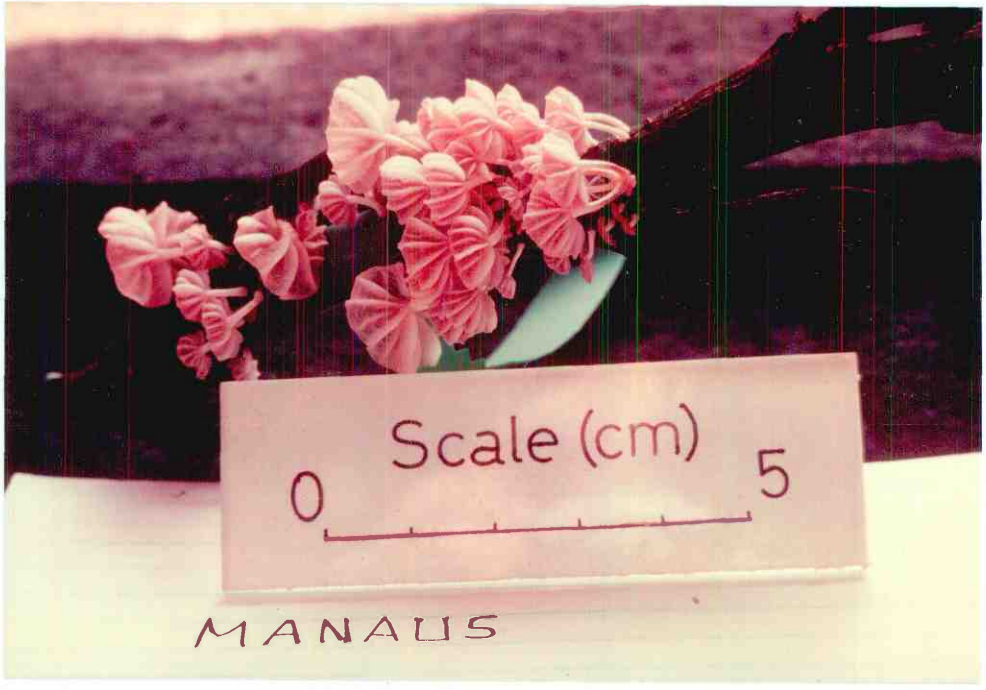


FIGURE I.5

The brooms which were transferred from regimes A, C and D to regime B at the end of 15 weeks showed different reactions in terms of basidiocarp production over the next 4 weeks. Those from regime A and C immediately produced flushes of basidiocarps whereas those from regime D did not produce any basidiocarp during this further period (Fig I.6).

The water content of brooms was assessed before they were placed in the cabinets by taking five samples, each c. 1.5 g, and drying at 105° C to constant weight. A similar procedure was used to determine the water content of brooms during wet and dry periods of each regime, 7 weeks after the start of the experiment. Here, four samples were taken at 3-hour intervals.

The water content of brooms from Castanhal and Manaus before they were placed in the cabinets was 13% (w/w), whilst that of brooms from Ouro Preto was 15%. The water balance in the brooms and its association with the humidity inside the cabinets, 7 weeks after the start of the experiment, is shown in Fig I.7. The various regimes led to different fluctuations in the water content of brooms, the size of which, with the exceptions of regime A, reflected in part the suitability of the regime for basidiocarp formation. Thus the difference between the maximum water content of the brooms during the wet period and the minimum content during the dry period was, respectively: A, 15.4%; B, 39.3%; C, 26.7% and D, 6.4% (w/w). The average water content in the brooms in each regime during the successive 24 h periods was, respectively: A, 20.2%; B, 36.3%; C, 47.6% and D, 76.6% (w/w).

The activity of *C. perniciosa* (and other micro-organisms which were visually assessed) in depleting cellulose and lignin was determined at the end of the experiment. This was done by measuring the solubility in hot 1% NaOH solution of broom material from each cabinet and comparing this with the solubility of comparable samples from untreated brooms. Two 2g samples

Fig I.6: Production of basidiocarps of *C. perniciosa* on brooms kept for 15 weeks (O) in regimes A, C and D and a further 4 weeks (●) on regime B.

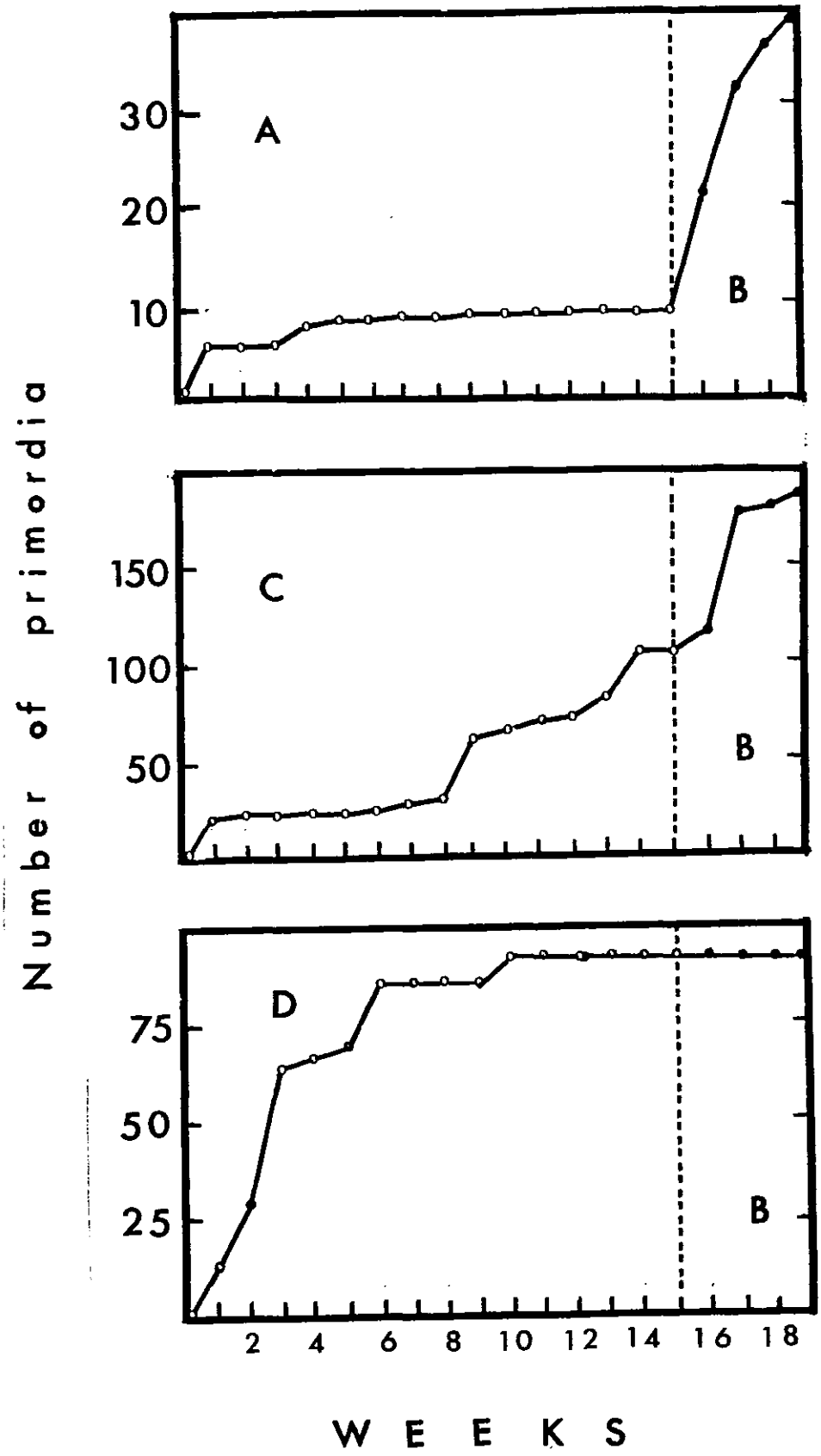

Number of primordia
30
20
10
A
B
150
100
50
C
B
75
50
25
D
B
2
4
6
8
10
12
14
16
18
WEEKS

FIGURE I.6

Fig I.7: Water balance in brooms subjected to different regimes of alternate wet (W) and dry (D) periods (O, r.h. inside cabinet; ●, water content of brooms).

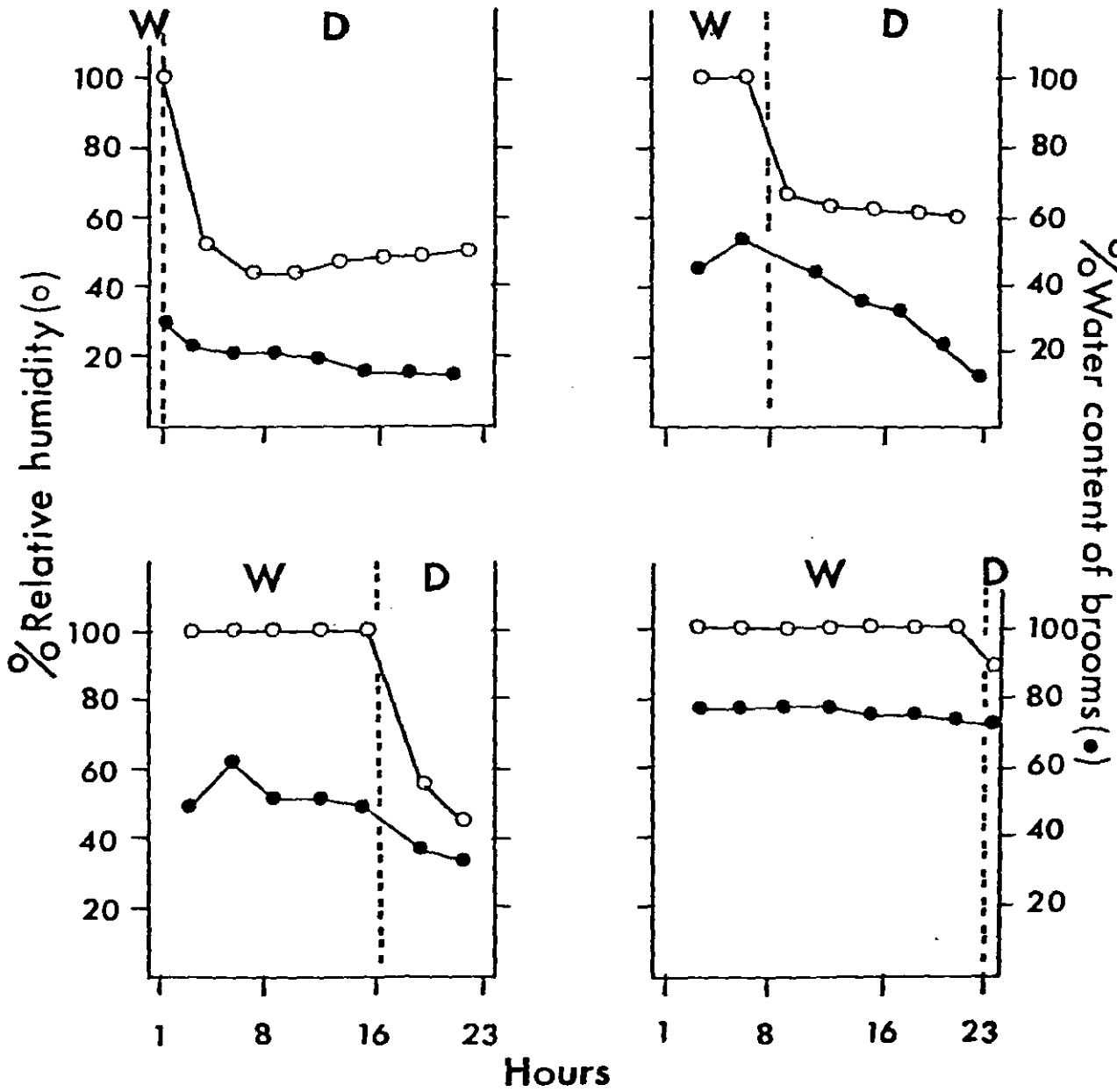

W
D
100
80
60
40
20
% Relative humidity (o)
% Water content of brooms (•)
1
8
16
23
Hours

FIGURE I.7

were taken from brooms of each region, these were previously air-dried and ground in a GRYPHON mill.

Each sample was placed in a 200 ml tall-form glass beaker and 100 ml 1% NaOH solution were added. The beaker was covered with a large watch glass and placed in a bath of boiling water. The mixture was stirred for c. 3-seconds at 10, 15 and 25 min. after placing in the water bath. After 1 h, the contents were filtered by suction through a tared nylon filter (45 µm mesh). The sawdust was first washed with hot (near boiling) water, then with 50 ml of 10% acetic acid and finally with hot water. Each nylon filter plus its treated broom material were dried to constant weight at 105° C. After cooling in a desiccator, each sample was weighed and the decline in non-soluble material was estimated from comparisons with the appropriate sample from untreated brooms (Anon., 1969).

The change in solubility of the broom wood in relation to the production of mature basidiocarps is shown in Fig I.8. The points are based on duplicate samples of brooms from each source within each regime. The formation of mature basidiocarps was associated in a general way with an increased solubility in NaOH (i.e. decline in non-soluble material), indicative of cellulose and lignin decomposition, wood from brooms in regime B being most soluble. However, there was a direct relationship between the two parameters only when the average values for the four regimes were plotted. The analytical method is normally used to estimate the degree of fungal decay that has taken place in wood but it would appear to be too crude to evaluate precisely the activity of *C. perniciosa* in brooms. Also, other fungi were present on brooms in some regimes (Table I.5), so these could have contributed to the losses in non-soluble material which were recorded. However, the apparent lack of other fungi on brooms in regime B where production of basidiocarps and loss of non-soluble material were greatest suggests that *C. perniciosa* degrades cellulose and/or lignin in the dead brooms.

Fig I.8: Relationship between solubility of broom wood in 1% NaOH and production of mature basidiocarps. (Data for brooms from three different sources in the four regimes, A, B, C and D - see legend to Fig I. 3. Average values for each regime indicated thus: ● A).

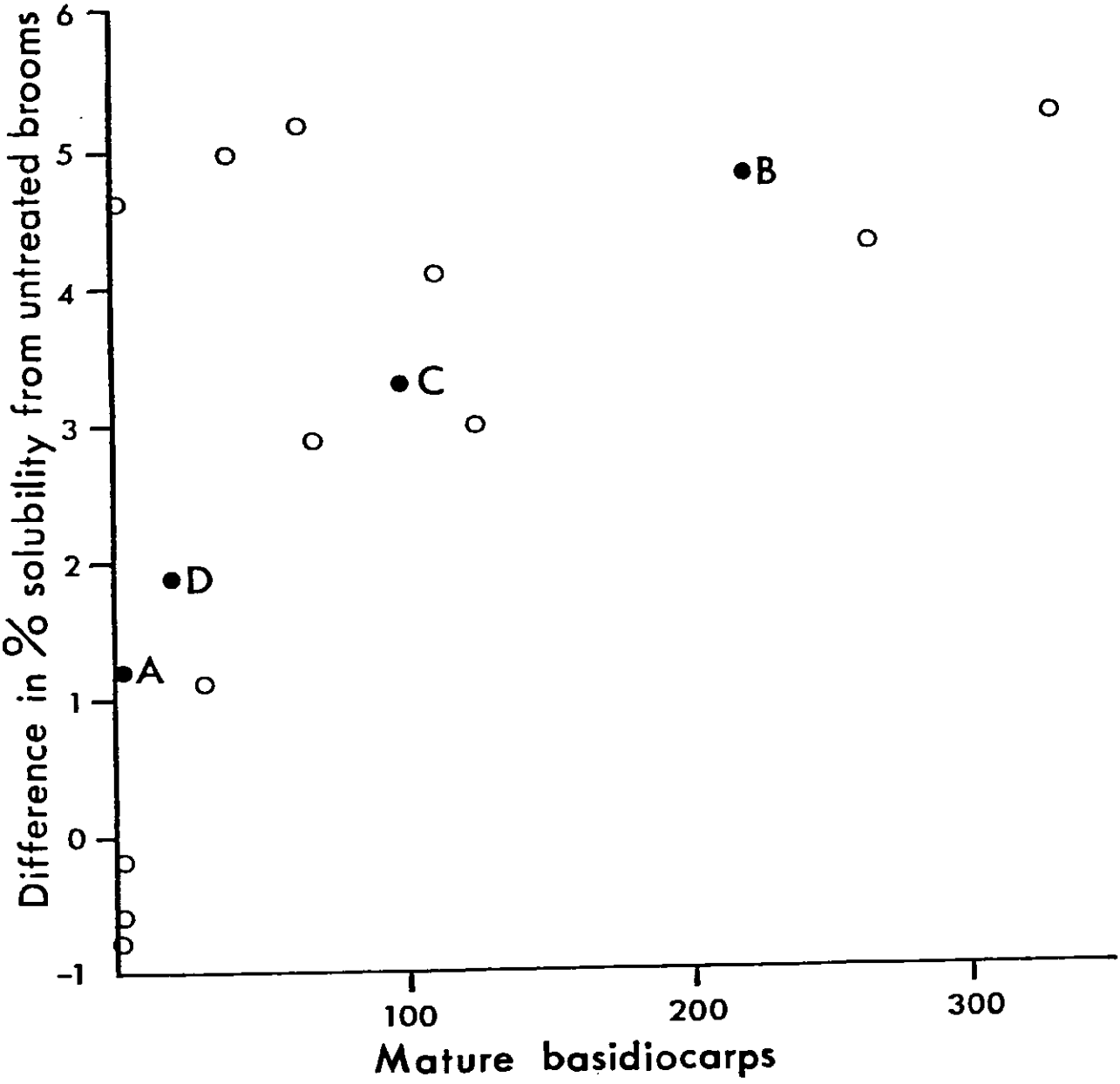

Difference in % solubility from untreated brooms
6
5
4
3
2
1
0
-1
A
B
C
D
100
200
300
Mature basidiocarps

FIGURE I.8

TABLE I.5

Micro-organisms on brooms subjected to different regimes of wet and dry conditions.

Regime (h wet/dry)	Micro-organisms[a]
1/23	None, except *C. perniciosa*
8/16	None, except *C. perniciosa*
16/8	*Aspergillus* sp.; *Rhizopus stolonifer.*; Actinomycete n.i.
23/1	*Aspergillus* sp.; *Penicillium* sp.; *Botriodiplodia theobromae* ; *Cylindrocladium* sp.; *Calonectria* sp.; *Coprynus* sp.; *Hohenbuelia barbatula*; Actinomycete n.i.

[a] Samples of the micro-organisms were taken from brooms and examined. *Hohenbuelia barbatula* was identified by Dr. D. Pegler of the Herbarium, Royal Botanic Gardens, Kew.

(b) Temperature

Electric heaters (Humex tubular heater) and a thermostat were installed in each of three cabinets so that with successive periods of 8 h wet and 16 h dry three temperature regimes were obtained : 20°-25° C ; 25°-30° C and 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 dry periods when cabinets were 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 (Fig I.9). All cabinets were housed in a room at 20° C illuminated (60 $\mu E/m^2/s$) during the dry period.

Ten, large brooms from the Castanhal region of Brazil were hung in each cabinet and the number of primordia and mature basidiocarps produced were counted weekly for 6 months. During this period, 20 mature basidiocarps were taken from each temperature regime, the colour and diameter of their pilei and length of stipes were assessed and measurements taken of 100 basidiospores. Temperature appeared to be an important factor determining not only the production of basidiocarps but also their morphological features, particularly colour. Most basidiocarps were produced on brooms at 20°-25° C , somewhat fewer were produced at 25°-30° C and none at all at 30°-35° (Table I.6). Comparisons of basidiocarp populations, using the Mann Whitney U Test, 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 $P = 0.0136$ and $P = 0.0008$ respectively. At 25°-30° C the stipes of basidiocarps were significantly longer ($P \leq 0.001$) and the caps were larger ($P \leq 0.05$) than those of basidiocarps produced at 20°-25° C (Table I.7). But, most strikingly, the pilei were white instead of usual crimson colour (Fig I.10).

(c) Light

Three cabinets were prepared, each at 20°-25° C and with a regime of

Fig I.9: Example of weekly thermohygrographs showing temperature (- - - -) and relative humidity (——) inside cabinets adjusted to a regime of 8 h wet/16 h dry at (a) 20°-25° C, (b) 25°-30° C, and (c) 30°-35° C.

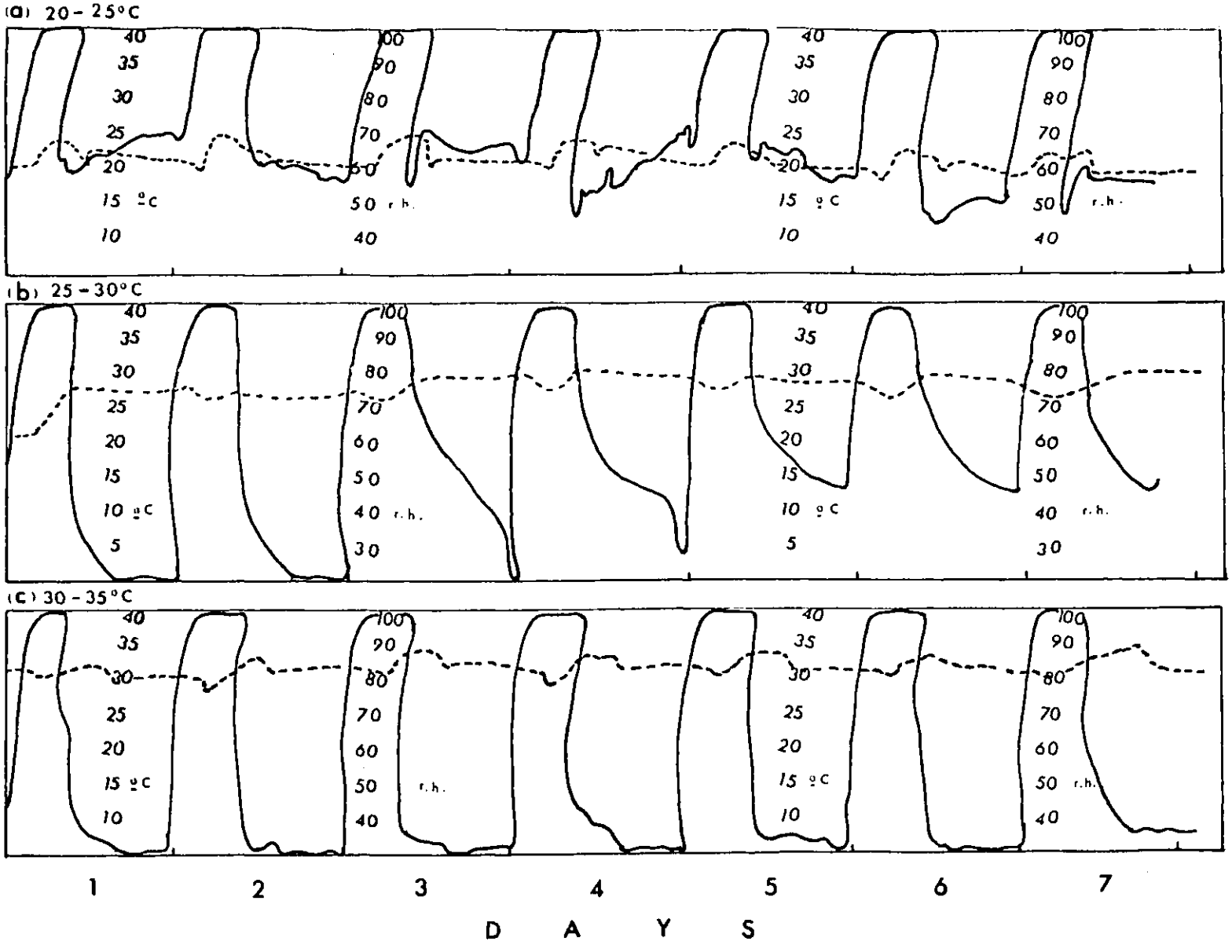


FIGURE I.9

TABLE I.6

Effect of temperature on the production of basidiocarps of *C. perniciosa*

Temperature regime (°C)[a]	Stage of Development	No. produced in successive 4-week periods.				Total	% maturing
		3[c]	4	5	6		
20-25°	Primordia	3	42	29	22[b]	96	
	Mature basidiocarps	1	33	28	13	75	79
25-30°	Primordia	10	19	13	1	43	
	Mature Basidiocarps	0	13	9	1	23	53
30-35°	Primordia	0	0	0	0	0	-
	Mature Basidiocarps	-	-	-	-	-	-

[a] Regime of 8 h wet/16 h dry

[b] Each figure is the number produced on 10 brooms over 4 weeks.

[c] No basidiocarps were produced at any regime in the first two 4-week periods.

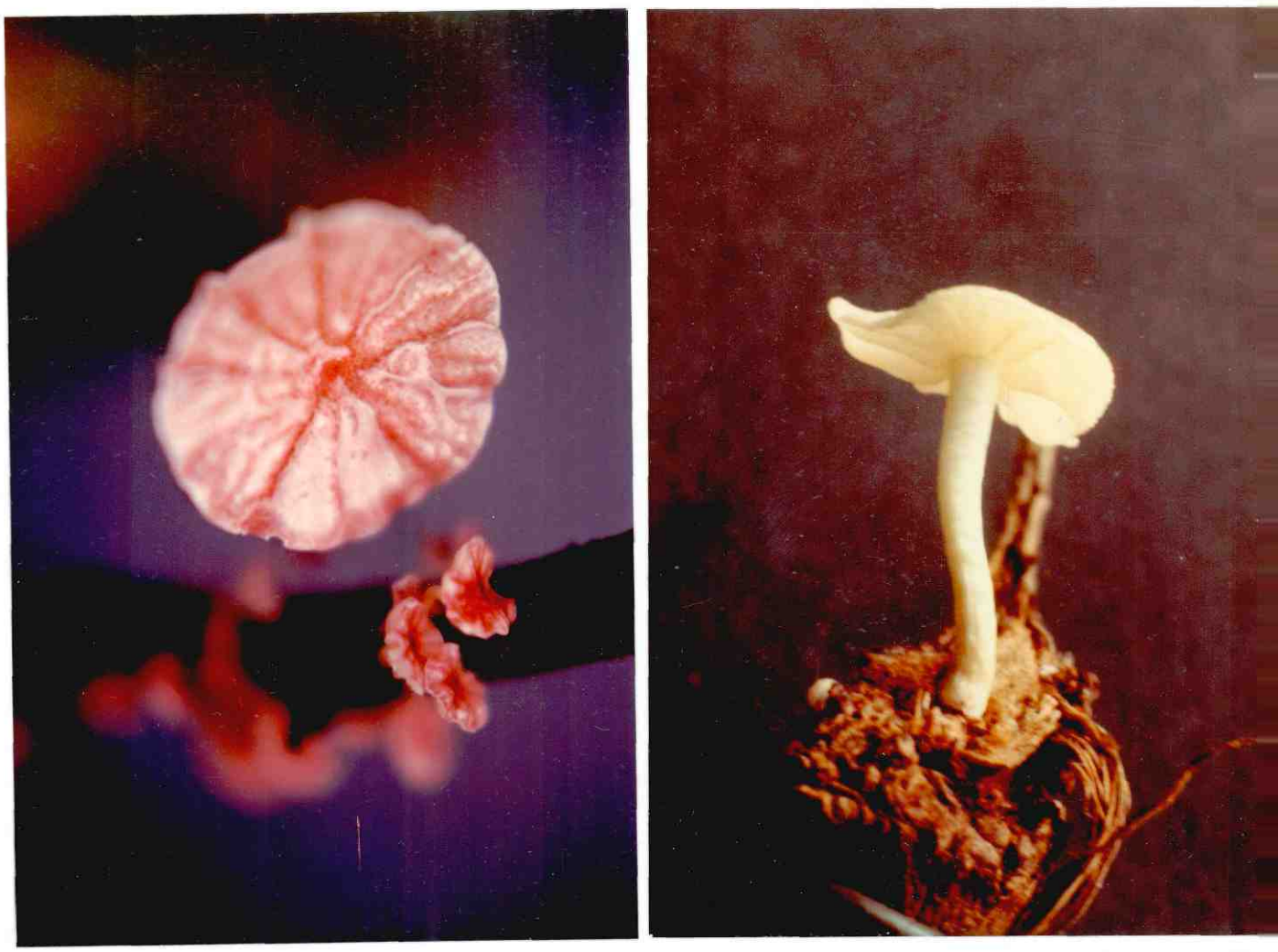
TABLE I.7

The effect of temperature on the morphology of basidiocarps and basidiospores of *C. perniciosa*[a]

Temperature regime (°C)	Pileus Colour	Pileus diameter (mm) Range	Mean	± S.D.	Stipe length (mm) Range	Mean	± S.D.	Basidiospore size (µm) Length	Breadth	length / breadth
20-25°	Crimson	4-22	10.6	± 4.8	6-11	7.7	± 1.5	11.6	6.4	1.8
25-30°	Whitish	6-12	8.0	± 1.3	7-12	10.8	± 1.4	11.9	6.5	1.8

[a] Means of 20 basidiocarps and 100 basidiospores in each temperature regime.

Fig I.10: Basidiocarps formed at: (a) 20°-25° C and (b) 25°-30° C. (Isolate from Castanhal, Brazil).



(a) (b)

FIGURE I.10

8 h wet and 16 h dry. These cabinets were kept in a room at 20° C, illuminated at 60 $\mu E/m^2/s$ for 8 h corresponding to the wet period. One cabinet was not changed in any way. Measurements with a Solartron 7040 light meter inside this cabinet indicated a light intensity of 10 $\mu E/m^2/s$. Two white fluorescent tubes (Atlas warmwhite super fine, 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 $\mu E/m^2/s$ during the wet period. 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 large brooms from the Castanhal region of Brazil were hung individually in each cabinet and the numbers of primordia and mature basidiocarps formed on them were counted weekly over 6 months.

The different light regimes affected basidiocarp production markedly. Significantly more primordia ($P = 0.0256$) and mature basidiocarps ($P = 0.0003$) were produced on brooms illuminated at 100 $\mu E/m^2/s$ than on those at 10 $\mu E/m^2/s$ (Table I.8) although the number of brooms producing basidiocarps was similar (15 & 14 respectively). 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. This suggests that light promotes the development of primordia and possibly their further growth.

(d) Cocoa cultivars

Ten, large brooms were collected in August 1981 from several trees of the following clones in the cocoa germplasm collection at Pichilingue, Ecuador: UF 168, UF 677, Sca 6, Sca 12, ICS 1, ICS 39, ICS 95, IMC 67, EET 400 and Catongo. These brooms were hung individually in cabinets at 20°-25° C with a regime of 8 h wet and 16 h dry on 7 November 1981. The cabinets were

TABLE I.8

Effect of light intensity on the production of basidiocarps by *C. perniciosa*.

Light intensity (μ E/m^2/s) during wet period[a]	Stage of development	No. produced in successive 4-week periods				Total	% maturing
		3[b]	4	5	6		
100	Primordia	5	48	94	18[c]	165	
	Mature basidiocarps	3	31	63	16	113	72
10	Primordia	0	21	22	11	54	
	Mature basidiocarps	-	8	16	3	27	50
Darkness	Primordia	0	0	5	0	5	
	Mature basidiocarps	-	-	1	-	1	20

a Regime of 8 h wet/16 h dry

b No basidiocarps were produced in any regime during the first two 4-week periods

c Each figure is the number produced on 20 brooms over 4 weeks.

kept in a room at 20° C, illuminated at 60 $\mu E/m^2/s$. The numbers of primordia and mature basidiocarps which developed on each broom were counted weekly for 7 months.

There were some differences in the production of basidiocarps on brooms from the different cultivars (Table I.9). In a general way the collection of brooms could be divided into three groups related to the mean number of mature basidiocarps produced per broom: > 10, Sca 6, Sca 12 and UF 168; 8-10, IMC 67, Catongo and EET 400; < 8, UF 677, ICS 39, ICS 95 and ICS 1. This separation appeared less clear when populations of basidiocarps produced on brooms of different cultivars were compared using the Mann Whitney U Test. However, the numbers of mature basidiocarps on brooms of Sca 6 were significantly greater ($P \leqslant 0.02$) than on brooms of all other clones except Sca 12. Zero values for four brooms of UF 168 imposed limits on the analysis and a comparison of this clone indicated a significant difference in total numbers produced on 10 brooms whereas the mean number of basidiocarps per producing broom (Table I.9) indicates no real difference.

(e) Age of broom

During 1981 groups of 10 brooms were labelled from their initiation within a commercial planting of cocoa hybrids (crosses between several cultivars) at Ouro Preto, Rondonia, Brazil, 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 on 2nd September in cabinets at 20°-25° C with a regime of 8 h wet and 16 h dry. The cabinets were housed in an illuminated room at 20° C as in (d) above. The time taken for the first primordium to appear and the total numbers of primordia and mature basidiocarps produced were assessed over 9 months.

As the broom takes approximately 1 - 2 months to become dry, the minimum period from this point to start the basidiocarp production which is known as

TABLE I.9

Basidiocarps produced on brooms of different cocoa clones from Pichilingue over 7 months.

Clone	Brooms with basidiocarps	Mean No. per producing broom	
		Primordia	Mature basidiocarps
Sca 6	10	26.4	21.0
UF 168	6	24.6	17.3
Sca 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

'dormant' period appears to be at least 3 months. This was clear with the brooms formed at 30 June (Table I.10).

The numbers of primordia and mature basidiocarps per producing brooms of different ages did not differ significantly when comparisons were made using the Mann Whitney U Test.

(f) Broom tissue

In the previous experiments most basidiocarps appeared to form at the nodes of the hypertrophied shoots within the dead broom. This was examined in a further experiment. Since nodes are areas from which leaves or side shoots arise and are then detached, 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 scapel; usually one at a node but two to three between nodes. In terms of wounding, there were three treatments: none; wounding at the node; wounding between nodes (internodes). Seven large brooms from Pichilingue, Ecuador, were used in each treatment and they were kept in cabinets at 20°-25° C with a regime of 8 h wet and 16 h dry as in (e). The numbers of mature basidiocarps were counted over a period of 9 months.

The results (Table I.11; Fig I.11) confirmed that most basidiocarps are produced at nodes, the differences in numbers at nodes and internodes being very highly significant ($P \leqslant 0.001$). 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 ($X^2 = 5.36$, $P \leqslant 0.1$). Therefore it appears that wounding did not affect their production.

Discussion:-

The results show that basidiocarps of *C. perniciosa* can be regularly and profusely produced if dried brooms are subjected to adequate regimes of alternating wet and dry conditions, temperature and light. In the most

TABLE I.10

Basidiocarp production on brooms of different ages (O. Preto isolate).

Broom formed (1981)	Age of broom (months)		Days from harvesting to first primordia		Mean No. basidiocarps per producing broom[a]	
	At harvesting	when placed in cabinet	Mean	Range	primordia	mature
27 March	4	5	88	59-158	31	24
28 April	3	4	105	59-191	25	22
28 May	2	3	109	89-188	29	21
30 June	1	2	136	93-196	23	18

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

TABLE I.11

Basidiocarps produced on brooms from Pichilingne at nodes and internodes with or without wounding.

Treatment[a]	Site of basidiocarps	No. produced in successive 4-week periods							Totals	X^2
		3[b]	4	5	6	7	8	9		
None	Node	20[c]	48	0	3	22	4	5	102	80.3^{***}
	Internode	1	6	0	0	1	0	0	8	
Wounds at node	Node	8	57	3	2	45	8	6	129	83.3^{***}
	Internode	7	4	0	0	6	1	0	18	
Wounds at internode	Node	9	51	2	11	33	5	5	116	86.8^{***}
	Internode	0	8	0	2	1	0	0	11	

a All brooms were kept at 20°-25° C with 8 h wet and 16 h dry

b No basidiocarps were produced on any broom in the first two 4-week periods.

c Each figure is the number produced on 7 brooms over 4 weeks.

*** X^2 significant at $P \leq 0.001$

Fig I.11: Brooms from Pichilingue, Ecuador, kept at 20°-25° C with a regime of 8 h wet/16 h dry, showing production of basidiocarps mainly at the nodes.

FIGURE I.11

favourable regime B (8 h wet, 16 h dry), basidiocarp primordia formed on 92% of the brooms and the numbers produced were over one hundred times more than in regime A (1 h wet, 23 h dry), ten times more than in regime D (23 h wet, 1 h dry) and more than twice those of regime C (16 h wet, 8 h dry). Clearly the degree of wetting and drying had a profound effect. Neither the extreme dry nor wet conditions of regimes A and D respectively favoured basidiocarps. Presumably in regime A the water content of the brooms (15% - 30%) was too low to support the extensive fungal growth which is a feature of basidiocarp development. In contrast, in regime D the surface of the brooms was colonized extensively by other micro-organisms and these appear to have had an adverse effect on *C. perniciosa*. This colonization by other micro-organisms was also observed in the system used by Suarez-Capello (1977) where brooms were laid on mesh within cabinets, rather than hung individually as in the present experiments. Further evidence that the two regimes affect *C. perniciosa* in different ways was obtained by transferring brooms after 15 weeks to regime B. Basidiocarps were produced on those previously in the dry regime A, as well as on those in the intermediate regime C but not on those from the wet regime D.

The water balance within the brooms is thus a most critical factor. One aspect of this could well be the degree to which the growth of *C. perniciosa* is favoured relative to that of other micro-organisms. It was an additional feature of brooms in regime B that they supported a population of basidiocarps virtually free of other micro-organisms (Table 1.5). However, it is also possible that the periodic reduction in water content of the brooms has other effects such as a direct stimulation of *C. perniciosa* itself or a re-orientation of the cellulose fibrils within the cocoa wood which facilitates their degradation by this fungus. Growth of *C. perniciosa* is associated with changes in the NaOH solubility of the wood which indicate some cellulose and lignin degradation. Degradative enzymes such as laccase, peroxidase, catecholase and cellulase

have been identified in culture filtrates of *C. perniciosa* (Lindeberg & Molin, 1949; Krupasagar & Sequeira, 1969), but their role in the colonization of cocoa tissue by this fungus is unknown. The occurrence of flushes of basidiocarps (Fig I.4) suggests that, as with the cultivated *Agaricus bisporus* (Turner, 1974), there are successive cycles of absorption of nutrients into the mycelium and their depletion by fruiting.

There have been few observations on the effects of light and temperature on fruiting of *C. perniciosa* (INIAP, 1972; Reyes & Reyes, 1976), but the present studies indicate that these factors are important, as they are with other basidiomycetes (Wilkins & Harris, 1946; Perkins, 1969; Manachere, 1980). When the water requirements are satisfied, temperatures between 20° and 25° C and a light intensity of 100 $\mu E/m^2/s$ are particularly favourable for basidiocarp formation. Temperatures over 30° C were inhibitory but such temperatures are not common for prolonged periods in regions of S. 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 species or at least as a variety of *C. perniciosa*. Few such variants have been found in nature. Pegler (1978) 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 temperatures in this range will affect basidiocarps in the field because these develop mainly during wet periods when, at least during the night, temperatures are lower. The failure of *C. perniciosa* to produce basidiocarps in extremely wet conditions and absence of light needs to be examined more closely, for this might be exploited in the field to control the flushes of basidiocarps on brooms detached from cocoa by pruning. It might also indicate likely micro-organisms for the biocontrol of *C. perniciosa*.

The fact that brooms from different cocoa clones at Pichilingue supported

different numbers of basidiocarps is of particular interest. Most basidiocarps were produced on brooms of Sca 6 and Sca 12 which in other experiments (Wheeler & Mepsted, 1982) proved susceptible to the Ecuadorian isolate of *C. perniciosa* but relatively resistant to the Brazilian pathotype. Clearly such material is unsuitable for Ecuador. On the other hand, the low numbers of basidiocarps on brooms from ICS I suggest that more use might be made of this clone.

Experiments on the age of broom suggest that a minimum period of 4 months is required after brooms form before basidiocarps can develop on them. This result agrees with that of Aranzazu (1981) for field conditions in Colombia. Such data are useful in planning sanitation programmes and will be discussed more fully later with other aspects of this section.

SECTION II

BASIDIOSPORE DISCHARGE AND GERMINATION

Introduction

Some aspects of the effect of 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 spores 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 mature, 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 between 15° and 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.

This section reports the results of three experiments related to basidiospore discharge and germination:

(a) germination and infectivity of basidiospores from basidiocarps induced under different conditions of light and temperature;

(b) the effects of temperature, relative humidity and light on basidiospore discharge; and

(c) the effects of temperature and light on basidiospore germination.

Experimental

(a) Germination and infectivity of basidiospores from basidiocarps induced under different conditions of light and temperature.

The main aim of this experiment was to assess if the pronounced effect of temperature and light on basidiocarp formation could also affect the germination and infectivity of basidiospores produced by them. The experiment compared basidiospores from basidiocarps produced at 20°-25° C with those produced at 25°-30° C (both at 10 $\mu E/m^2/s$ inside the cabinets) and, similarly, compared basidiospores from basidiocarps produced at 10 $\mu E/m^2/s$ with those produced at 100 $\mu E/m^2/s$ (both at 20°-25°). The experiments from which these basidiocarps were derived are described in Section I. Two treatments within this present experiment are thus similar.

Five basidiocarps of the isolate of *C. perniciosa* from Castanhal (Brazil) were used to supply basidiospores. For germination tests excised pilei were stuck to the lid of a petri dish with Vaseline and basidiospores were allowed to deposit onto distilled water in the base for 4 h at 25° C under white fluorescent lights. Drops of 0.1% cotton blue-lactophenol were added to prevent further germination which was then assessed on a minimum of 150 basidiospores from each source.

For infectivity tests, basidiospores were allowed to deposit on plates of 1% water agar for a few (< 5) min. Pieces, 1-2mm across, were cut from the spore print and applied to developing buds on 2-month-old cocoa seedlings (Na 32 x UIT 1) in the glasshouse, a technique similar to that described by Evans & Bastos (1980). For each source of basidiospores, five seedlings were inoculated. The extent of broom formation was determined 8 weeks after inoculation as the maximum diameter of the swollen shoot.

The results (Table II.1) showed that basidiospore germination and infectivity

TABLE II.1

Basidiospore germination and stem swelling induced on cocoa using spores from basidiocarps produced under different temperature and light regimes

	Conditions on which basidiocarps were induced				Uninoculated control
	Temperature (°C)		Light Intensity ($\mu E/m^2/s$)		
	20-25	25-30	10	100	
Basidiospore germination (%)[a]	83	76	87	77	-
Stem diameter[b] (cm)	0.7	0.8	0.7	0.8	0.4

[a] Germination in distilled water, 4 h after deposition at 25° C; each figure is based on a minimum of 150 basidiospores.

[b] Each figure is the mean of five replicates of 2-month-old seedlings of Na32 x UIT1, 8 weeks after inoculation.

did not differ significantly in any treatment.

(b) 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 (10.15 $\mu E/m^2/s$) and in the dark. Thus, there were in all 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. Each was set up as follows.

Fifteen newly-formed basidiocarps of an isolate of *C. perniciosa* from Castanhal (Brazil) 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 x 10 cm) with Vaseline so that the gills faced the bottom of the box. The base of each box had 25 compartments (2 x 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 one 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, covered plastic box (17.5 x 11.5 cm) containing 100 ml of a solution of glycerine (51%, 44%, 32.5% or 12.5% glycerine in distilled water) 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.

The mean numbers of basidiospores discharged in the 10 experiments are shown in Table II.2 and an analysis of variance on the results is shown in Table II.3a. Overall, temperature and humidity affected basidiospore discharge

TABLE II.2

Effects of temperature, relative humidity and light on basidiospore discharge.

%r.h.	Thousands of basidiospores deposited in 4 h[a]										Mean
	10°		15°		20°		25°		30°C		
	L	D	L	D	L	D	L	D	L	D[b]	
80	13.2	10.7	156.0	166.7	357.7	332.0	584.0	482.7	56.0	48.0	232.0
85	12.0	8.0	262.7	281.3	476.0	424.0	574.7	480.0	62.7	70.7	269.6
90	14.7	13.3	217.3	240.0	430.7	437.3	445.3	388.0	113.3	93.3	244.3
95	6.7	4.0	190.7	170.7	348.0	342.7	376.0	365.3	10.7	38.7	186.4
100	17.3	12.0	225.3	184.0	370.7	368.0	304.0	300.0	166.7	100.0	216.8
Mean	12.8	10.4	210.4	208.5	395.2	380.8	448.8	403.2	81.9	70.1	-

a Each figure is the mean of three replicates

b Basidiocarps either in the light (L) at an intensity of 10.15 $\mu E/m^2/s$ or in the dark (D).

significantly ($P < 0.001$) but light/dark did not. There was also a very highly significant ($P < 0.001$) temperature x humidity interaction.

The effects of temperature and humidity were examined further by regression analysis with basidiospores as dependent variable. The corresponding analyses of variance are indicated in Table II.3b. The regression of basidiospores on temperature was very highly significant ($P < 0.001$) and was best represented by the equation, $y = 140.6x - 29.97x^2 - 124.88$ (Fig II.1). Separate regressions of basidiospores on temperature at each of the five relative humidities were basically similar. All were very highly significant ($P < 0.001$) and were described best by quadratic equations. The results generally indicated an optimum temperature for basidiospore discharge near 25° C

The overall regression of basidiospores 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 regressions were significant. The regression coefficients were negative but that for basidiocarps in the light (- 17.97) was not significantly different from that for basidiocarps in the dark (- 12.0).

(c) Effects of temperature and light on basidiospore germination

Basidiospores were allowed to deposit from basidiocarps of an isolate of *C. perniciosa* from Castanhal (Brazil) onto microscope slides covered with a thin layer of 1% water agar. Three pilei were suspended over each slide to give three separate spore prints. Each slide was then placed in a covered plastic box (17.5 x 11.5 cm) on wet blotting paper and incubated at one of the following temperatures: 10°, 15°, 20°, 25° and 30° C with or without light (10 $\mu E/m^2/s$). After 3 h a drop of cotton blue-lactophenol was placed on each print. Germination was assessed from counts of basidiospore within five fields (x10 x40) in each spore print giving over 500 basidiospores in each regime. The length of 20 germ-tubes was also measured in each regime.

TABLE II.3

Effect of temperature, relative humidity and light on basidiospore discharge: analyses of variance

(a) Main factors and their interactions

	Source of variation	Df	SS	MS	F-ratio
A	Temperature	4	253303	63325	294.3***
B	Light	1	384	384	1.79
C	Humidity	4	5678	1419	6.60***
AB		4	803	200	0.93
AC		16	16367	1022	4.75***
BC		4	196	49	0.23
ABC		16	1852	115	0.54
	Sampling error	100	21517	215	-

(b) Regressions

Source of variation		Df	SS	MS	F-ratio
Temperature	T	1	23144	23144	45.82***
	T^2	1	202708	202708	401.32***
Temp. at r.h.	80%	2	49469	24734	28.87***
	85%	2	65323	32661	43.23***
	90%	2	48836	24418	80.44***
	95%	2	39383	19691	91.0***
	100%	2	28037	14018	65.21***
Humidity	H	1	1855	1855	0.92

Table II.3 (b) cont.....

Table II.3 (b) cont.....

Source of variation		Df	SS	MS	F-ratio
Humidity at	10° C	1	0.13	0.13	0.02
(Light)	15° C	1	300	300	0.88
	20° C	1	145	145	0.41
	25° C	1	9684	9684	19.15***
	30° C	1	537	537	2.65
Humidity at	10° C	1	0.83	0.83	0.18
(Dark)	15° C	1	108	108	0.50
	20° C	1	1.63	1.63	0.004
	25° C	1	4320	4320	24.61***
	30° C	1	97	97	1.4

*** F-values significant at $P < 0.001$

FIGURE II.1 : Regression of basidiospores deposited on temperature

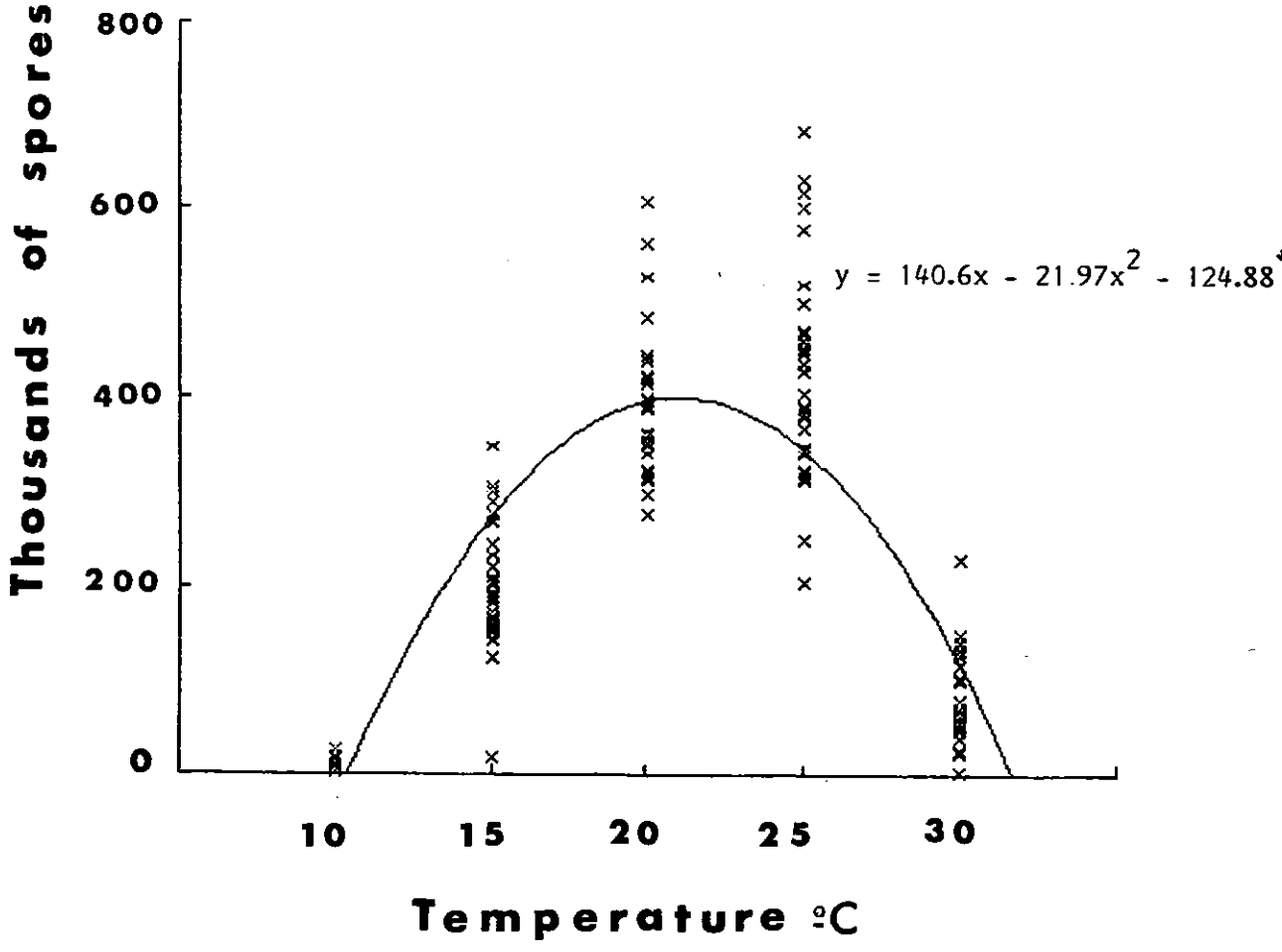


FIGURE II.1

*Original scale: Temperature, 10°=1; 15°=2; 20°=3; 25°=4; 30°=5
Spores: 200=50; 400=100; 600=150; 800=200.

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 II.4). Light did not affect germination or germ-tube growth.

Discussion

The data presented in this section provide clear evidence of an appreciable effect of temperature on basidiospore discharge (Table II.2 and Table II.3), germination and further growth of germ-tubes (Table II.4). Basidiospores were liberated when temperatures ranged from 10°-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 experiment temperature effects, which were considerable, were confounded with days on which the experiments were done and thus with the particular crop of basidiocarps. Generally, over the range 10°-30° humidity had little effect on basidiospore discharge. Although as a factor in the overall analysis humidity was significant, the regression of basidiospores on humidity was not significant. However, there was a significant temperature x humidity interaction which related to two experiments at 25° C, one in the light and one in the dark (Table II.3). 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 is affecting the process. They agree with the suggestion of Baker & Crowdy (1943), based on field observations, that a relative humidity c. 85% might be most favourable for basidiospore discharge.

Light had no significant effect either on the discharge of basidiospores or their germination but the intensity used (10.15 $\mu E/m^2/s$) was low.

TABLE II.4

Effects of temperature and light on the germination of basidiospores and germ-tube length.

Temperature °C	Mean % germination[a] ± S.E.		Mean germ-tube length (μm)[b] ± S.E.	
	Light[c]	Dark	Light	Dark
15	17.3 ± 1.1	16.1 ± 1.2	4.8 ± 0.4	5.0 ± 0.4
20	74.1 ± 2.9	71.7 ± 2.4	29.6 ± 2.5	25.1 ± 3.2
25	85.7 ± 1.7	90.0 ± 0.0	79.3 ± 3.5	80.3 ± 3.4
30	57.6 ± 2.1	58.7 ± 1.4	67.9 ± 3.0	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 Each figure is based on 20 replicates

c Basidiospores in light (10 $\mu E/m^2/s$) or in the dark

SECTION III

INFECTION, SYMPTOM DEVELOPMENT AND BROOM FORMATION

Introduction

Apart from the type and age of tissue involved, the vigour of the plant and genetic background, there are no references in the literature concerning the effect of climatic factors such as temperature on infection of cocoa by *C. perniciosa*, the development of symptoms and broom formation. Also, the effect of different pathotypes of the fungus on symptom devlopment has been reported only recently (Wheeler & Mepsted, 1982).

This section describes experiments which investigated the effects of temperature on infection and symptom development on seedlings inoculated at the hypocotyl. The types of brooms induced by pathotypes of *C. perniciosa* from Brazil and Ecuador after inoculating buds of different cocoa cultivars were also assessed.

Experimental

(a) Effect of temperature on infection and symptom development

Seeds of a Nacional x Sca 12 cross were imported from Pichilingue, Ecuador. After removing the testas, these seeds were placed on wet absorbent tissue in a seed germination tray (37 cm x 24 cm, Leithen Valley Plastics Ltd) and kept at 25° C for 4 days.

Basidiospores of an Ecuadorian (Pichilingue) isolate were collected by allowing them to deposit on water agar from excised pilei for c. 3 min at 20°-25° C. Small (2-3mm) pieces were cut from the spore prints and these were placed on the hypocotyls (one piece per hypocotyl) of 60 germinated seeds. Fifteen seeds with inoculated hypocotyls and fifteen with uninoculated hypocotyls were placed in each of four seed germination trays in constant

temperature rooms at 15°, 20°, 25° and 30° C, one tray per room. After a further 48 h, the seedlings were planted in pots (8.5 x 8.5 cm, 10 cm deep) of compost (50% John Innes no. 3, 25% sand, 25% moss peat), with a pair of seedlings, one inoculated, one uninoculated in each pot. The pots were placed on wet capillary matting on a tray (75 cm x 35 cm) under a polyethylene cover and kept in the same CT room for 8 weeks.

The maximum diameter of each stem base was measured after 4 weeks and the increase in stem base of an inoculated plant compared to its corresponding control was used as an indicator of infection and the extent of symptom development. Inoculated plants with no obvious indication of infection were kept in the pots for a further assessment 4 weeks later.

The incubation period varied according to the temperature, being shortest at 30° C and longest at 15° C. At 30° C three plants showed symptoms 12 days after inoculation and 100% of the plants kept in this temperature showed a very clear swelling 13 days later. In contrast, the first symptom on plants kept at 15° C appeared only 27 - 30 days after inoculation and on most plants, visual swelling was detected only after 60 days. At 20° C and 25° C most plants showed symptoms 20 - 30 days after inoculation (Table III.1).

Temperature also had a profound effect on the extent of stem base swelling. At 30° C the mean increase of stem base diameter over corresponding controls was about 10 times greater than that found at 15° C. The differences between the increase in stem base swelling at 30° C and that at all other temperatures were significant($P \leqslant 0.001$). At 25° C the increase in stem diameter was greater than at 15° C ($P \leqslant 0.01$) but the differences in swelling at 20° C and 15° C was significant only at the 5% level (Table III.2 and Fig III.1).

Plants kept at 15° C grew very slowly. Also it seems that at this temperature the activity of the fungus, especially its initial growth, was extremely reduced. Despite these unfavourable conditions, the fungus was still able to

TABLE III.1

Effect of temperature on symptom development on Nacional x Sca 12 cocoa seedlings inoculated on the hypocotyl with an isolate of *C. perniciosa* from Pichilingue, Ecuador.

Days after inoculation	No. of plants with symptoms (out of 15)			
	15°	20°	25°	30°C
12	0	0	0	3
15	0	0	3	7
20	0	5	9	13
25	0	5	10	15
27	1	7	11	15
30	7	11	14	15
60	12	13	14	15

TABLE III.2

Effect of temperature on infection and stem base swelling of Nacional x Sca 12 seedlings, 4 weeks after inoculation of the hypocotyl with an isolate of *C. perniciosa* from Pichilingue, Ecuador.

° C	Plants infected (out of 15)	Increase in stem diameter (cm) over corresponding control		
		Replicates	Mean[a]	S.E.
15	7	0.05, 0.1, 0.07, 0.13, 0.06, 0.13, 0.05	0.08 A	± 0.07
20	11	0.4, 0.75, 0.35, 0.46, 0.17, 0.25, 0.25, 0.24, 0.20, 0.22, 0.22	0.32 AB	± 0.06
25	14	0.4, 0.4, 0.32, 0.58, 0.47, 0.4, 0.4, 0.2, 0.33, 0.5, 0.95, 0.64, 0.24, 0.4	0.48 B	± 0.05
30	15	1.24, 0.46, 0.64, 0.49, 0.54, 1.0, 0.8, 0.94, 0.63, 0.69, 1.03, 1.03, 0.49, 0.76, 1.03	0.79 C	± 0.05

[a] Figures with no letter in common are significantly different ($P \leq 0.01$)

Fig III.1 Effect of temperature on seedlings of cocoa (Nacional x Sca 12), 4 weeks after inoculating them on the hypocotyl with an isolate of *C. perniciosa* from Ecuador (Pichilingue). In each pair, the inoculated seedling is on the left; the control is on the right.

FIGURE III.1

induce some swelling, but at a very low rate compared with other regimes. Clearly temperatures between 25° and 30° C are the most favourable for the host/pathogen association and consequently for the most dramatic symptom expression.

(b) Effects of inoculum source and cocoa cultivar

(i) Experiment 1

Seeds of Sca 6, EET 400, and UF 168 cultivars were imported from Pichilingue, Ecuador. These were germinated in trays as described in (a) above and then planted in pots of compost. The pots were kept on capillary matting in a glasshouse at c. 25° C with a relative humidity not less than 80%. When the cotyledons had expanded fully the shoot was cut back to induce the development of the two buds at the cotyledon node.

Dead witches' brooms were obtained from the three cultivars at Pichilingue. These were hung individually in cabinets at 20° - 25° C with 8 h wet and 16 h dry to induce the formation of basidiocarps (Section I). Basidiospores were collected on agar and small blocks of the spore print were used as inocula (Section II). An agar block with basidiospores was applied to one bud only at the cotyledon node; the other bud was excised. Each inoculated plant was covered with a polyethylene bag for 2 days and then left uncovered on the glasshouse bench.

Fifteen plants of each cocoa were inoculated with each source of basidiospores, giving for any one cocoa 45 inoculated plants. For each cocoa/ inoculum combination there were also fifteen uninoculated plants which were paired with those inoculated, giving a total of 270 plants in the experiment.

Broom formation was assessed in two ways, 7 weeks after inoculation. The maximum diameter of hypertrophied shoots (Fig III.3) on inoculated plants and of corresponding healthy shoots on its paired control (Fig. III.2)

were measured. The difference between these measurements was used as an indicator of infection and symptom development. Brooms formed on inoculated plants were classified into two types: 'simple brooms', which characteristically consisted of a swollen shoot with few side branches (Fig III.3a) and 'proliferating brooms' where many swollen branches developed giving the form of broom typically seen in the field (Fig III.3b).

The overall infection (61%) in this experiment was not particularly high; symptoms developed on 71% of Sca 6, 51% of UF 168 and 60% of EET 400 seedlings which were inoculated. However, a chi-squared test indicated that the proportion of brooms formed on the three cocoas was the same for each source of inoculum (Table III.3).

Data on the type of broom formed are shown in Table III.4. These indicate that, over all the cultivars, the source of inoculum had no significant effect on the type of broom (Table III.4a). However, significantly more ($P \leqslant 0.05$) proliferating brooms were formed on Sca 6 from the three inocula than simple brooms, suggesting that proliferation is a feature particularly evident on this cultivar (Table III.4b).

The source of inoculum did, in some instances, affect the amount by which inoculated shoots became swollen relative to the controls. On Sca 6 inocula from brooms of Sca 6 and UF 168 induced significantly more ($P \leqslant 0.05$) swelling than inoculum from brooms of EET 400 (Table III.5). Similarly, on UF 168 inocula from these sources also induced significantly more ($P \leqslant 0.01$) stem swelling than that from brooms of EET 400. In contrast, on EET 400 there were no significant differences in the amounts of swelling induced by the inocula from the three sources.

(ii) Experiment 2

Seeds of Sca 12, Sca 6, Catongo and IMC 67 cultivars were imported from Itabuna, Bahia, Brazil. These were germinated in trays, planted in pots

FIGURE III.2 Sca 6 seedling with healthy normal shoots.

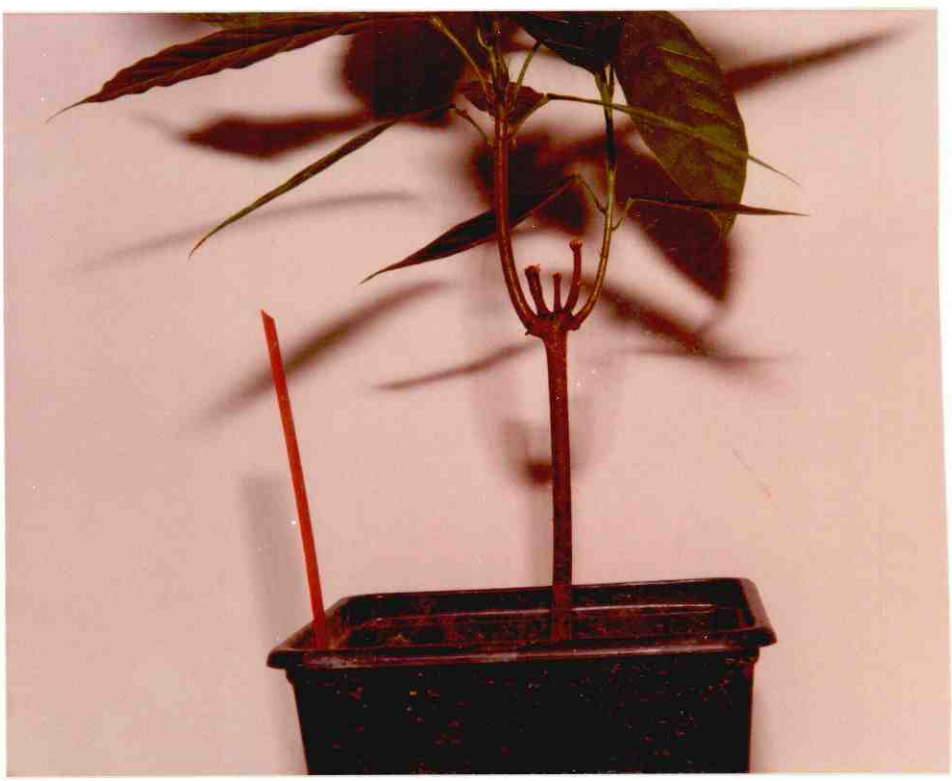


FIGURE III.2

Fig III.3 Simple (a) and proliferate (b) brooms produced by *C. pernicíosa* (Ecuador isolate) on seedlings of respectively EET 400 and Sca 6, seven weeks after bud inoculation.

(a)

(b)

FIGURE III.3.

TABLE III.3

Number of seedlings infected (out of 15) using inoculum of *C. perniciosa* from different cocoa clones at Pichilingue, Ecuador.

Cocoa inoculated	Cocoa from which inoculum derived			Totals (out of 45)
	Sca 6	UF 168	EET 400	
Sca 6	12	10	10	32
UF 168	8	8	7	23
EET 400	10	10	7	27
Totals (out of 45)	30	28	24	82 (Grand total)

TABLE III.4

Associations between the type of broom formed and (a) the source of inoculum and (b) cocoa cultivar.

(a)

Inoculum Source	Type of Broom		X^2
	Proliferating	Simple	
Sca 6	15	15	n.s
UF 168	15	13	n.s
EET 400	14	10	n.s

(b)

Cocoa Cultivar	Type of Broom		X^2
	Proliferating	Simple	
Sca 6	22	10	4.53*
UF 168	12	11	n.s
EET 400	10	17	n.s

* Significant at $P \leqslant 0.05$

TABLE III.5

Maximum diameter (cm) of inoculated shoots, relative to the corresponding controls, on three cocoa cultivars inoculated with *C. perniciosa* from three sources (Pichilingue, Ecuador).

Inoculum Source[a]	Increase in diameter (cm) over control[a]		
	Sca 6	UF 168	EET 400
Sca 6	0.26 A	0.33 A	0.33 A
UF 168	0.30 A	0.36 A	0.33 A
EET 400	0.18 B	0.16 B	0.46 A

[a] Based on the numbers of infected seedlings shown in Table III.3
Values in the same column with no letter in common are significantly different.

and the shoot cut back to induce growth of the buds at the cotyledon node as in Experiment 1 above. Dead witches' brooms were obtained from Ouro Preto (Brazil) but the cocoa hybrid was not named. Basidiocarps were induced on these brooms and inoculum of basidiospores was obtained on water agar. For inoculations with *C. perniciosa*, a small block of agar was applied to one cotyledon bud; the other bud was excised. The increase in diameter of the inoculated shoot 8 weeks after inoculation was compared with the shoot diameter of a corresponding control. The extent of broom formation was also assessed by counting the number of branches over 1 cm long within each broom. Different numbers of seedlings of the four cocoa cultivars were available for this experiment as detailed in Table III.6.

Table III.6 and Fig III.4 show the different reactions of the cultivars in terms of infection, branching and swelling. The percentages of Catongo and IMC 67 seedlings which became infected were both significantly higher ($P \leqslant 0.01$) than the corresponding values for Sca 6 and Sca 12. The same level of significance was found when either the number of branches or the increase in stem diameter was compared. No significant differences were found in any comparisons between Catongo and IMC 67 or between Sca 6 and Sca 12.

Discussion

Witches' broom symptoms produced on cocoa seedlings after artificial inoculation of the meristems are expressed in different ways depending on the organ inoculated.

The complex branching of shoots formed after bud inoculation at the cotyledon node which is typical of a 'broom' is not an easy system to measure quantitatively and thus to evaluate the effect of temperature on the pathogen-host interaction. In contrast the stem base swelling which results from an inoculation of the hypocotyl with *C. perniciosa* provides a much simpler measure of the effect of the pathogen on its host. Clear effects of temperature on

TABLE III.6

Reaction of cocoa cultivars 8 weeks after inoculation with an isolate of *C. perniciosa* from Ouro Preto, Brazil.

Cultivars	No. plants		% infection	Mean increase (cm) in diameter of main shoot[a]	Mean No. branches/broom
	Inoculated	Infected			
Catongo	62	46	74.2 A	0.18 A	7 A
IMC 67	59	37	62.7 A	0.20 A	6 A
Sca 6	64	20	31.3 B	0.11 B	3 B
Sca 12	69	12	27.5 B	0.11 B	2 B

[a] Compared with corresponding control. Figures in the same column with no letter in common are significantly different ($P \leq 0.01$).

FIG III.4 Swelling on cocoa seedlings, 8 weeks after bud inoculation with an isolate of *C. perniciosa* from Ouro Preto, Brazil. Left to right: IMC67, Catongo, Sca 6, Sca 12.

FIGURE III.4

symptom development were determined using this system. Increase in temperature from 15° C to 30° C accelerated symptom development (Table III.1) and the extent to which symptoms developed (Table III.2; Fig III.1). Comparisons of mean stem base diameter of uninoculated and inoculated plants (Fig III.5) show a remarkable contrast. The stem base diameter of uninoculated plants showed only a small response to temperature with a peak at 25° C; that of inoculated plants increased almost linearly with temperature. The mean % increase in stem diameter of inoculated plants over controls was 95% at 25° C and 182% at 30° C. This abundance of affected tissue which is clearly seen in Fig III.1 suggests that this system could be valuable for the study of host-parasite interactions. In such studies the choice of cocoa and isolate of the fungus is clearly important. An isolate of *C. perniciosa* from Ouro Preto, Brazil caused extensive brooming on Catongo and IMC 67 but not on Sca 6 or Sca 12 (Table III.6). It did not even infect the latter two cocoas well and where it did there was comparatively little stem swelling or branching in the broom (Fig III.4). In contrast an isolate of *C. perniciosa* from Pichilingue, Ecuador caused extensive brooming on Sca 6 (Fig III.3b). There were also some indications that the type of broom formed (proliferating broom) was a feature of Sca 6 rather than the isolate used.

Nevertheless, there were also indications that even within one locality there may be differences in isolates of *C. perniciosa* which influence the extent to which they induce brooms on the same type of cocoa. Thus inoculum from EET 400 at Pichilingue did not induce as much swelling on Sca 6 and UF 168 as did inocula taken from these two cocoas (Table III.5).

The relevance of these results to other aspects of this research will be discussed later.

Fig. III.5 Mean stem base diameter of cocoa seedlings inoculated (●) with an isolate of *C. perniciosa* from Pichilingue, Ecuador and of corresponding uninoculated (O) plants, at different regimes of temperature. Measurements taken 4 weeks after inoculation.

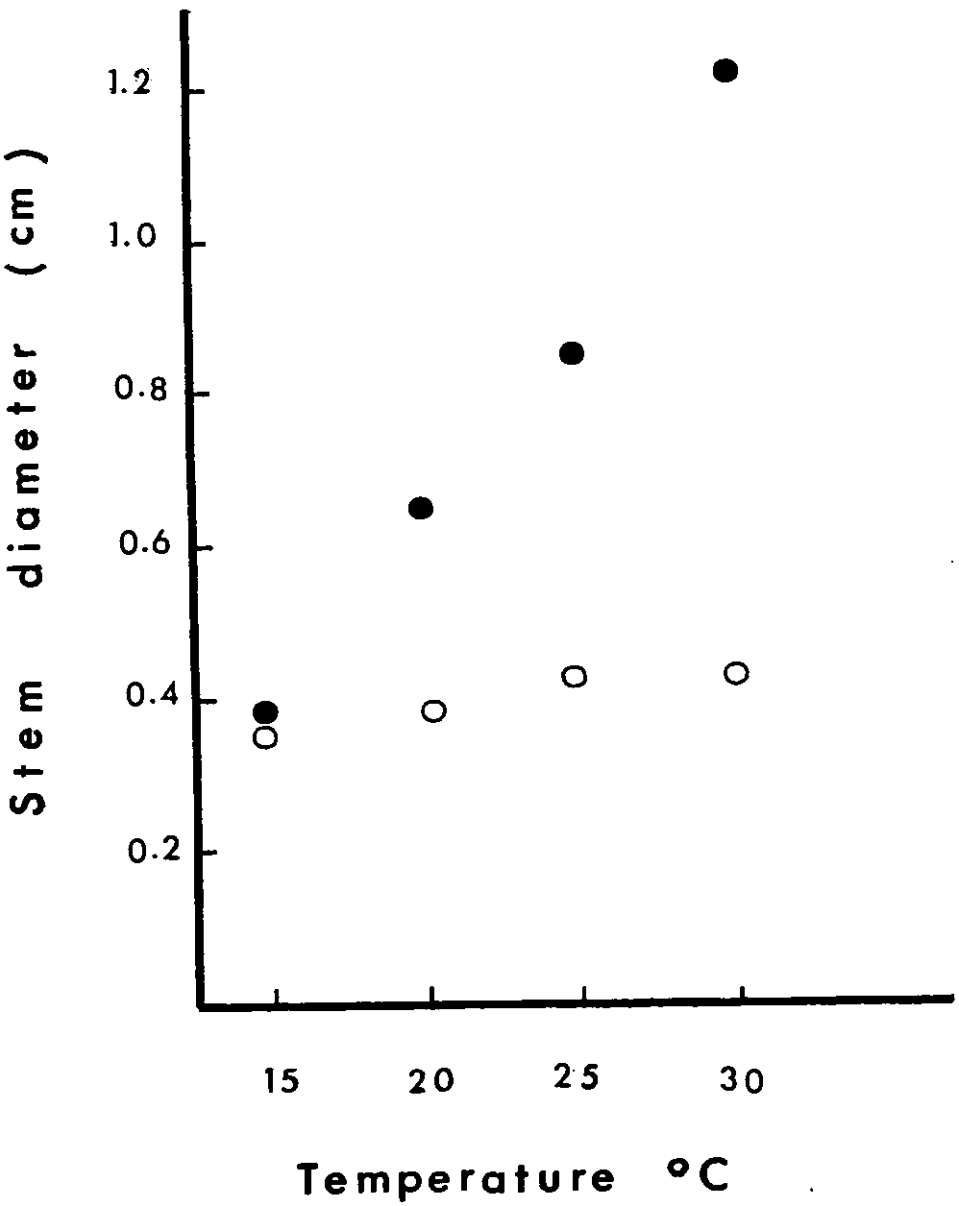


FIGURE III.5

SECTION IV

GROWTH OF *CRINIPELLIS PERNICIOSA* IN BROOMS AS DETERMINED BY GLUCOSAMINE ASSAYS OF FUNGAL BIOMASS

Introduction

In the experiments described in Section I, the ability of *C. perniciosa* to develop on dead brooms was estimated directly by counting basidiocarps. Its ability to grow in living cocoa tissue was measured indirectly by assessing the host reaction in the experiments described in Section III. Although these indicators of fungal activity and growth were appropriate for the aims of the experiments they give little indication of the ability of the fungus to colonize either dead or living broom tissue. This would require an estimation of fungal biomass within the broom.

In many instances an estimation of fungal biomass can be achieved by microscopic examination of serial sections of diseased tissue but it is difficult to use this method for *C. perniciosa* in dead brooms. The hyphae are thin (1-4 μm wide) and are not readily seen within the dark tissues of the host. In other instances some indication of fungal colonization can be obtained by plating standard amounts of diseased tissue on suitable agar media. It is more difficult to obtain quantitative measurements of fungal biomass in this way and the method cannot be applied directly to the fungus in green brooms since the growth obtained on agar is like that seen in dead brooms (saprophytic stage) and not the form characteristically observed within the green broom (parasitic stage). Several methods have been developed in an attempt to achieve a reliable quantitative estimate of fungal biomass in infected plant tissues. These are based on hydrolysis of infected tissues and subsequent determination of its chitin content (Arima & Uozumi, 1967; Lung-Chi Wu & Stahmann, 1975; Ride & Drysdale, 1971; Ride & Drysdale, 1972; Swift, 1973). With the exception of Phycomycetes, chitin, a polymer of β - 1 - 4 - N - acetyl - D - glucosamine, is a component of fungal cell-walls varying from

< 1 to 25% of mycelium dry weight (Aaronson, 1965; Bartnicki-Garcia, 1968; Sturgeon, 1974). As the component amino sugars are found only in trace amounts in plants (Racusen & Foote, 1974), their determination provides a convenient means of estimating the amount of fungus in infected tissues. The analytical procedure most frequently used for the determination of hexosamine are based on methods developed by Elson & Morgan (1933) and Morgan & Elson, (1934). These involve the condensation of hexosamine products with acetylacetone which, after reaction with Enrlich's reagent, develop a pink colour with an absorbtion peak at 530 mμ . Another method, developed by Tsuji *et al.* (1969) involves acid hydrolysis and deamination of hexosamines with nitrous acid to yield the aldehyde 2,5-anhydromannose which reacts with 3-metyl-2-benzothiazolone hydrazone hydrochloride (MBTH) in presence of Fe Cl_3 producing an intense blue colour with maximum absorbance at 650 mμ . Variations in these methods have been introduced by several workers (Ride & Drysdale, 1972; Swift, 1973; Toppan, 1976). Their accuracy in determining fungal biomass in plant tissue depends upon obtaining a reliable conversion factor (CF) relating glucosamine content to units of mycelium dry weight. This can be done by growing mycelium separate from the substrate under study.

This section is concerned with the assessment of fungal biomass in living and dead cocoa tissues previously infected with *C. perniciosa*. The main aim was to relate the amount of fungus in the host to the production of basidiocarps and to the extent of symptom expression, under different regimes. This was achieved first by adapting the existing techniques to the cocoa/witches' broom system and, second by assessing the fungal biomass in different broom tissues as well as in brooms subjected to different regimes of temperature and wet/dry conditions.

Methods and Experimental:

(a) - The development of analytical methods

(i) *in vitro*: - The fungus was grown in the following medium:-

malt extract, 25 mg; casein hydrolysate, 1.5 mg; glucose, 20 g; yeast extract, 1.0 mg; thiamine hydrochloride, 100 µg and distilled water, 1000 ml. The pH was adjusted to 5.5 and 50 ml volumes were dispensed into 250 ml conical flasks before sterilization in an autoclave at 125° C for 15 min. Each flask was inoculated with a 4 mm disk cut from the margin of a fresh colony of *C. pernicíosa* grown on V-8 juice-agar medium. The flasks were incubated at 25° C under fluorescent light for 2, 3 and 4 weeks. Isolates from Chigorodo (Colombia), Pichilingue (Ecuador), Ouro Preto (Brazil) and Trinidad were used and the mycelium dry weight as well as the glucosamine content were assessed after each period of growth in three replicates. The mycelium was collected over a nylon sieve (53 micro mesh), washed several times with distilled water and homogenized in water (10-15 ml; Omnimixer speed 8, 30 sec.). Three 2 ml portions from the mycelial suspension were transferred to 15 ml centrifuge tubes and centrifuged at 1500 g for 10 min. The supernatant was removed, the residue mixed with 3 ml 6N HCl and heated in 10ml glass-stoppered volumetric flasks in a boiling water bath for 6 h. The hydrolysates were filtered (Whatman no.1 Filter paper), the filtrates evaporated in a rotary-evaporator immersed in a water bath at 45° C and then dried further in a desiccator under reduced pressure in the presence of KOH pellets to remove excess HCl. The residue was resuspended in 3 ml distilled water and evaporated again. The final volume was made up to 4 ml with distilled water after neutralization with 1 N NaOH and 1 % $KHSO_4$, using 0.5% phenolphthaleine in ethanol as indicator (Tsuji *et al.*, 1969).

Three samples of 5 ml from the mycelial suspension were placed in tared aluminium foil boats, and dried to constant weight at 100° C.

The glucosamine determination was carried out using basically the methods of Tsuji *et al.* (1969) and Ride & Drysdale (1972). Aliquots of 1.5 ml of the hydrolysates were mixed with 1.5 ml of 5 % $NaNO_2$ and 1.5 ml of 5 % $KHSO_4$

(w/v) and shaken for 15 min. The mixture was centrifuged at 1500 g for 5 min. before an aliquot (1.5 ml) of the supernatant was taken and placed into a test tube. To each tube, 0.5 ml of 12.5 % $NH_4SO_3NH_2$ (w/v) was added and shaken for 5 min. Then, 0.5 ml of 0.5 % MBTH (w/v) was added and the tubes heated in a boiling water bath for 3 min. After cooling, 0.5 ml of 0.5 % Fe Cl_3 (w/v) was added. The intensity of the blue colour which developed was read 30 min. later in a Beckman DB spectrophotometer at 650 mμ . A blank and glucosamine standards (glucosamine hydrochloride, Sigma Chemical Company) were included in each determination. The conversion factor (CF) was determined by assessing the amount of glucosamine per unit of dry mycelium in samples taken from the same mycelial suspension and was expressed as μg glucosamine per mg dry mycelium.

Mycelium dry weight increased with age of the culture but not linearly, growth per day being greater between days 15 and 21 than between days 21 and 28 (Table IV.1). Total glucosamine also increased with age of culture so that, generally, values for the conversion factor were not markedly different. Comparisons of the values for the conversion factor obtained for the four isolates, using a t-test for small samples (nine values per isolate) indicated no significant differences between the means or variances of the samples. The data from the four isolates were therefore pooled and the regression of mycelium dry weight (y) on glucosamine content (x) plotted using the 36 pairs of values from the experiment (Fig IV.1). The regression was highly significant ($P \leqslant 0.001$), the regression coefficient and its standard error being 0.124 ± 0.007. There were thus two possible methods for calculating mycelium dry weight from determinations of glucosamine in infected tissues: by using the regression equation, $y = 0.124x - 3.759$ or the overall, mean conversion factor, 8.52. Since for low values of glucosamine the regression equation gave small negative values for mycelium dry weight, the mean conversion factor of 8.52 was chosen.

TABLE IV.1

Mycelium dry weight (mg), total glucosamine content (μg) and the corresponding conversion factor (CF = μg glucosamine per mg dry weight of mycelium) of four isolates of *C. perniciosa*

Isolate	Mycelium Days			Total glucosamine Days			CF Days		
	15	21	28	15	21	28	15	21	28
Chigorodo	59.8[a]	118.5	157.4	516.1	977.6	1116.0	8.63	8.25	7.09
Pichilingue	52.3	131.7	152.2	450.3	1110.2	1266.3	8.61	8.43	8.32
Trinidad	51.7	150.3	201.1	451.3	1352.7	1566.6	8.73	9.00	7.79
O. Preto	51.1	145.3	207.8	475.2	1349.8	1789.2	9.30	9.29	8.61

[a] Each value is the mean of three replicates

FIGURE IV.1 : Regression of mycelium dry weight of four isolates of *C. perniciosa* on glucosamine :

△ , Chigorodo; ▲, Pichilingue; □ , Trinidad; ■, Ouro Preto.

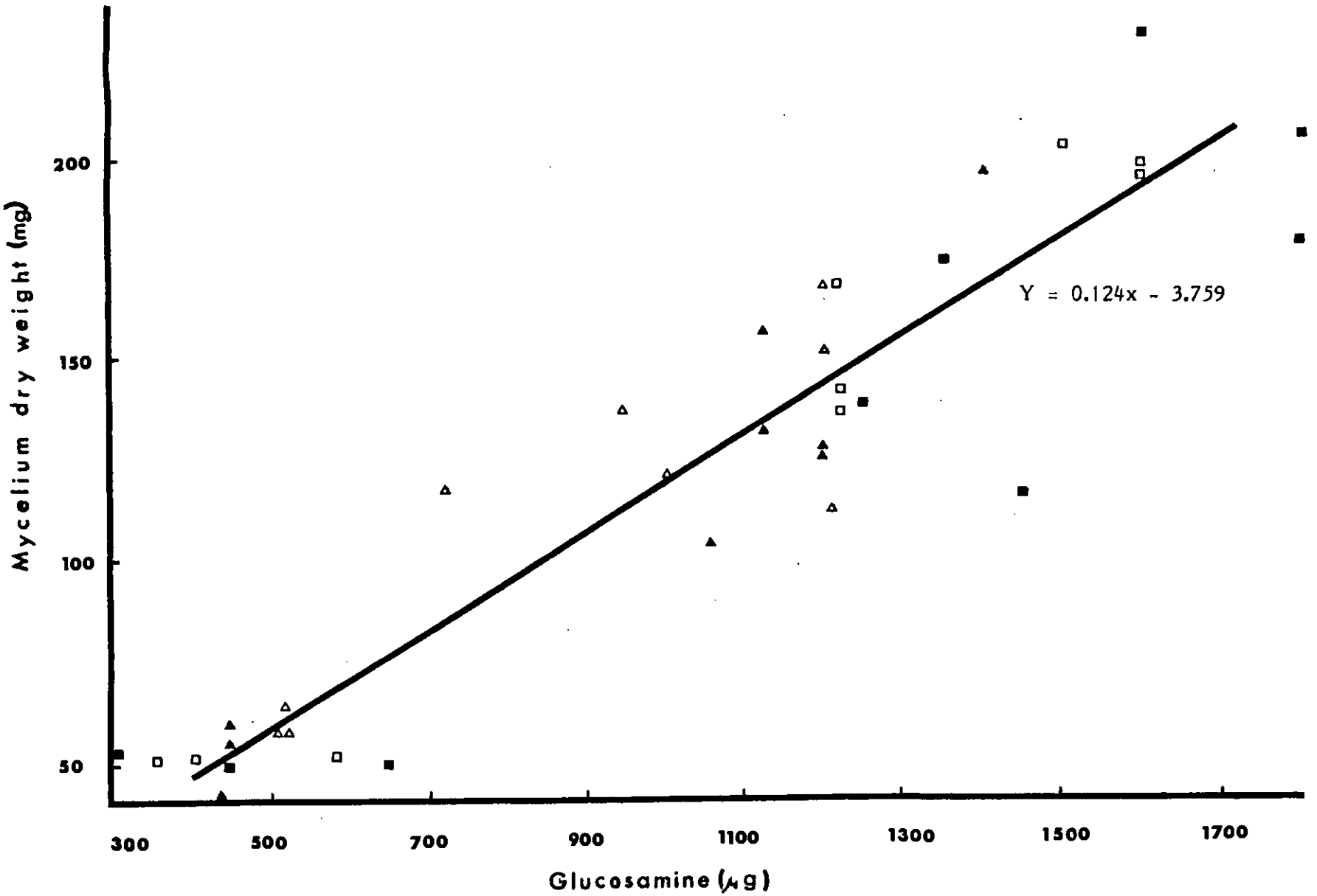

Mycelium dry weight (mg)
200
150
100
50
Y = 0.124x - 3.759
300
500
700
900
1100
1300
1500
1700
Glucosamine (μg)

FIGURE IV.1

(ii) *in vivo* :- The greatest difficulty in determining glucosamine in cocoa tissues was the lack of information regarding the use of existing methods for woody tissues such as dead brooms. Extracts from dead brooms are dark, even after centrifugation, and this masks the colour produced by the reaction in the glucosamine assays. Problems related to difficulties in grinding the material properly before hydrolysis and interference of other chromogens were only solved after many attempts with various techniques. The method of Swift (1973) which is concerned with wood tissue decayed by fungi was extremely helpful specially for sampling the material properly.

Small pieces (c. 0.5 cm) of broom tissue (green, dying or dead) were cut and dried at 80° C. After drying the material was ground in a GRYPHON mill, the sawdust was collected and passed through a 50 µm-mesh sieve. Samples (25 mg) of the fine particles were mixed with 3 ml of 6 N HCl and transferred to 10 ml glass volumetric flasks. After pre-soaking the material for c. 3 h in the acid at room temperature (c. 20° C), the flasks were sealed and hydrolysis was carried out in a boiling water bath for 6 h. The hydrolysates were allowed to cool, then were filtered (Whatman no. 1 Filter paper) and pipetted onto column of cation exchange resin (Dowex 50, 200-400 mesh) prepared as described by Boas (1953). The columns were washed with 10 ml distilled water to remove non-absorbed material and the amino sugars were eluted with 2N HCl. The volume of acid necessary for complete elution of 100 µg of glucosamine was found to be c. 15 ml (Table IV.2) so that this volume was used in all determinations. The eluted material was evaporated as previously described and its glucosamine content was determined by the colorimetric method already described for the fungus alone. After use, the columns were treated with 12 ml 2 N NaOH and regenerated with 15 ml 2 N HCl. Before the columns were used again, 4 ml 2N HCl were run through to remove small amounts of resin which may go into solution on standing.

TABLE IV.2

Glucosamine recovered from Dowex 50 (200-400 mesh) column with 20 ml 2N HCl as effluent liquid[a]

Fraction (2 ml) eluted	Glucosamine recovered[b]	
	μg	%
1st	0.0	0.0
2nd	1.8	1.6
3rd	36.8	34.0
4th	43.9	40.3
5th	17.5	16.1
6th	7.0	6.4
7th	1.8	1.6
8th	0.0	0.0

a A total of 100 μg glucosamine hydrochloride (Sigma Chemical Company) in 10 ml distilled water was pipetted onto the column

b Each figure is the mean of two replicates

The cation exchange columns removed almost 100% of the interfering substances which made impossible any reading with untreated hydrolysates. Some components other than glucosamine probably still remained in very small amounts in the material eluted from the columns, since hydrolysates from healthy tissues gave a pale green-blue colour with the reagents. Comparisons with glucosamine standards indicated low values for hexosamines of c. 1.0 µg/25 mg dry matter compared with values of 9.4 - 87.5 µg/25 mg dry matter for hydrolysates from infected tissues so the method was considered satisfactory for the purpose of this study.

(b) Fungal biomass in different tissues of brooms

A mature plant of the cocoa cultivar Na32 x UIT1, grown in the glasshouse at Silwood Park, was inoculated with an isolate of *C. perniciosa* from Pichilingue, Ecuador. Three green brooms and three brooms which were beginning to die were collected 16 and 19 weeks respectively after inoculation. Samples were taken from the cortex and wood (all tissues other than cortex) of these brooms both at nodes and internodes. Three replicate samples of each type were used for glucosamine assay following the procedure already described and glucosamine content was converted to mycelium dry weight using the Mean conversion factor. The same methods were used to examine the distribution of *C. perniciosa* in three dead brooms collected from the cultivar IMC 67 at Pichilingue and then subjected to a regime of 8 h wet/16 h dry in a cabinet at Silwood Park (Section I). When examined these brooms were about 64 weeks old.

Table IV.3 shows the mean values obtained for relative amounts of mycelium in the cortex and wood of nodes and internodes within the three types of broom. Overall, the fungal biomass was greater in the nodes than internodes. The ratios, mycelium dry weight node/internode for green, dying and dead brooms were, respectively, 1.4, 1.2 and 1.2. The corresponding ratios

for the particular tissues examined were generally similar: for the cortex, 1.2, 1.2 and 1.1; for the wood, 1.6, 1.3 and 1.4. However, within nodes and internodes the relative amounts of mycelium in the cortex and wood differed with age of the broom. In green brooms there was significantly more ($P \leqslant 0.05$) fungus in the wood than in the cortex at both nodes and internodes; in dying and dead brooms fungal biomass was greater in the cortex. This is reflected by the ratios, mycelium dry weight cortex/wood for nodes and internodes respectively in the three types of broom: green brooms, 0.4 and 0.6; dying brooms, 1.1 and 1.3; dead brooms, 1.3 and 1.6. Values for fungal biomass, expressed as % of the total dry matter, ranged from 4.6% in green brooms to 40% in dead brooms.

(c) Fungal biomass in dead brooms subjected to different regimes of wetting and drying

Brooms were collected in Ouro Preto (Brazil) from plants of the open-pollinated cocoa cultivar SIAL 169. The estimated age of these brooms were 58 weeks. Brooms from this sample were hung in cabinets at Silwood Park, three brooms per cabinet, and subjected to one of the following regimes: 1 h wet/23 h dry; 8 h wet/16 h dry; 16 h wet/8 h dry; 23 h wet/1h dry. After 10 weeks, a sample from each broom (cortex and wood) was prepared for assay of glucosamine content, as described previously, from which mycelium dry weight was determined (Table IV.4). No basidiocarps developed on the brooms.

Although there were some differences in the amounts of fungal biomass in brooms kept in the different regimes (Table IV.4), only in brooms subjected to 16 h wet/8 h dry was the amount of fungus significantly greater ($P \leqslant 0.05$) than in the rest.

(d) Fungal biomass in brooms formed at different temperatures

Dead brooms (mainly swollen stem bases) were obtained from the experiment

TABLE IV.3

Mean amounts of mycelium (μg dry weight/mg dried broom) as determined by glucosamine assays, in brooms induced by *C. perniciosa* from Pichilingue, Ecuador.[a]

(a) Green brooms, Na 32 x UIT1

	Cortex	Wood	Mean	
Node	54.0	125.9	90.0	S.E. ± 5.72
Internode	46.4	78.7	62.6	
Mean	50.2	102.3		
		S.E. ± 10.77		

S.E. for body of table: cortex vs. wood at same site, ± 15.23; node vs. internode for same tissue, ± 17.53

(b) Dying brooms, Na 32 x UIT1

	Cortex	Wood	Mean	
Node	210.0	182.9	196.5	S.E. ± 7.39
Internode	180.6	136.3	158.5	
Mean	195.3	159.6		
		S.E. ± 9.32		

S.E. for body of table: cortex vs. wood at same site, ± 13.19; node vs. internode for same tissue, ± 16.83

(c) Dead brooms, IMC 67

	Cortex	Wood	Mean	
Node	404.4	303.8	354.1	S.E. ± 4.20
Internode	355.9	223.8	289.9	
Mean	380.2	263.8		
		S.E. ± 5.54		

S.E. for body of table: cortex vs. wood at same site, ± 7.83; node vs. internode for same tissue, ± 9.82

[a] For details of brooms see text. Means based on three replicates of each tissue at each site.

TABLE IV.4

Amounts of mycelium in brooms of SIAL 169 from Ouro Preto (Brazil), subjected to different regimes of wetting and drying.

Regime (h wet/dry)	Amount of mycelium (as µg dry wt/mg dried broom)[a]	
1/23	246.2	A
8/16	331.3	A
16/8	416.6	B
23/1	337.4	A

[a] Each figure is the mean of three replicates; figures with no letter in common are significantly different ($P \leq 0.05$).

described in Section III in which seedlings of the cocoa cultivar Nac x Sca 12 were inoculated on the hypocotyl with an isolate of *C. perniciosa* from Pichilingue and kept at 15°, 20°, 25° or 30° C (pp. 58 to 63, Fig III.1). The samples were taken 20 weeks after inoculation. Glucosamine was assayed and mycelium dry weight was estimated from these assays as described previously.

Table IV.5 shows that fungal biomass was significantly greater ($P \leq 0.05$) per unit weight of broom, in brooms developed at 30° C than in those developed at 15° and 25°. There were no other significant differences.

Discussion

Previous studies of the growth of *C. perniciosa* in brooms have been limited to the examination of stained sections and observing its hyphae within tissues. No techniques have been developed for determining the amount of this fungus in brooms.

The estimation of fungal biomass by assaying glucosamine content of infected tissues was examined in this Section. A technique was developed which enabled clear extracts to be obtained from dead brooms, suitable for recording colour development in hydrolysates treated with MBTH in the presence of ferric chloride. These relied on appropriate comparisons based on the growth of the fungus in a synthetic medium.

Such an approach has been criticized because the proportion of chitin (from which glucosamine is derived) to weight of mycelium is affected, amongst other things, by the environment in which the fungus grows (Sharma *et al.*, 1977) and for *C. perniciosa* the environment of a synthetic medium is obviously different from that of its natural habitat, a broom on a cocoa tree. Nevertheless, for a situation in which *C. perniciosa* is the predominant microorganism, at least initially, i.e. in the broom, the method would appear to be

TABLE IV.5

Amounts of mycelium in brooms induced at different temperatures on seedlings of Nac x Sca 12, by an isolate of *C. perniciosa* from Pichilingue, Ecuador.

Temperature °C	Amount of mycelium (as µg dry wt/mg dried broom)[a]	
15	267.0	A
20	306.9	AB
25	302.7	A
30	355.9	B

[a] Each figure is the mean of three replicates; figures with no letter in common are significantly different ($P \leq 0.05$).

of some value for comparing amounts of fungal biomass. Extending the method to green brooms is more open to criticism because the fungus cannot be grown on synthetic media in its parasitic form. These limitations require that the results of this section be interpreted with some caution. But some points of interest emerge. Clearly the fungus is not distributed evenly throughout dead brooms but is more abundant at nodes than internodes and, at these sites, is more abundant in the cortex than in the other tissues (Table IV.3c). Such a distribution was indicated in other experiments (Section I, Table I.11) by the greater production of basidiocarps at nodes.

The results suggest that in green brooms also, fungal development is greater at the nodes. This is possibly related to the greater meristematic activity and growth at these sites. However, in these brooms there is apparently more fungus in the tissues inside the cortex than in the cortex itself (Table IV.3a).

Because relationships between glucosamine content and amounts of mycelium were established by growing the saprophytic form of the fungus on a synthetic medium, comparisons of the amount of fungus and its distribution within green, dying and dead brooms must be made with particular caution. Observations by other workers (Pegus, 1972; Evans, 1980) indicate that as the broom dies the fungus changes to its saprophytic form. It could be speculated that the saprophytic mycelium is more efficient in synthesizing chitin than the parasitic mycelium and this could result in higher values of glucosamine per unit dry weight. If this were so, comparisons between mycelial biomass in the different types of broom would not be valid. However, the substantial amounts of glucosamine in dying brooms compared with green brooms from the same cultivar do suggest that there is a real increase in fungal biomass. The significant differences between the amounts in the cortex and wood at similar sites (Table IV.3 a & b) suggest also that as the broom dies the fungus

rapidly colonizes the cortex from the underlying tissues prior to forming its basidiocarps.

Dead brooms in this particular experiment were kept in a regime of 8 h wet/16 h dry in which virtually no fungus other than *C. perniciosa* develops (Section I, Table I.5). Therefore, changes in fungal biomass within brooms of a particular type can be ascribed to *C. perniciosa*, even if there is some doubt about the absolute amounts.

Comparisons of fungal biomass in brooms subjected to different regimes are less easy to interpret because other fungi develop in some of them, particularly in the regime of 23 h wet/1 h dry (Section I, Table I.5). Therefore, it is perhaps not surprising that there were few differences in total fungus biomass between the four regimes. The largest amount occurred in brooms kept in a regime of 16 h wet/8 h dry. This is interesting because most basidiocarps of *C. perniciosa* matured in this regime (Section I, Table I.2), suggesting that it particularly favoured the build-up of mycelium in the dead broom.

A high temperature of 30° C also appeared to favour the development of *C. perniciosa* in the swollen stem bases on seedlings of Nac x Sca 12 inoculated at the hypocotyl with this fungus. However, the amount of mycelium was not closely related to the amount of stem swelling observed in this experiment (Section III, Table III.2). There could be two reasons for this: the response of the parasitic mycelium to temperature in terms of chitin synthesis could be different to that of the saprophytic mycelium or the synthesis of growth regulators like gibberellic acid which are assumed to be responsible for the abnormal growth in brooms (Bastos & Andebhan, 1981) is adversely affected by low temperature.

GENERAL DISCUSSION

There are two features of witches' broom disease which make its control more difficult than other diseases of cocoa. First, the meristems are most vulnerable to infection. Thus, besides pod losses which arise directly from the infection of young pods and indirectly through infections of the flower cushions, successive infections of vegetative growth year after year, resulting in the formation of brooms, lead to a rapid build-up of the disease. Often in such a situation the more vigorous the growth of the plant, the greater are the chances of damage by this disease.

Second, the biology of *C. perniciosa* is complex. It appears to have two distinct forms of mycelium, having different functions in the life cycle - the establishment of infections on cocoa and the exploitation of the hypertrophied tissues which result from this process for the production of its basidiocarps.

Control of the disease has so far relied on three main approaches: sanitation, the use of chemicals and resistant cocoas. The removal and burning of brooms have long been advocated but sometimes these practices are uneconomic especially when the price of cocoa is low. Also, there are few precise data to indicate the effect of removing brooms on the rate at which, subsequently, new infections arise. Chemicals have been applied mainly to two different targets - to the dead brooms.in attempts to eradicate the fungus or at least to suppress the formation of its basidiocarps and to the young flush growth to protect it from infection. Neither approach has particularly found favour or been especially successful. Protection of flushes of new growth which are produced at several times throughout the year presents many practical problems and the use of chemicals generally is uneconomic in some areas. So far, no systemic fungicides have been found to be effective in

suppressing broom formation but this approach requires further exploration. So, too, does the use of biological control agents such as the fungus *Cladobotryum amazonense* which parasitizes the basidiocarps of *C. perniciosa* (Bastos *et al.* 1981). The use of cocoa resistant to *C. perniciosa* also remains only a long-term possibility especially since the Scavina cultivars which were effective in Trinidad have proved to be highly susceptible to the pathotype of *C. perniciosa* in Ecuador (Chalmers, 1972).

Lack of enough basic research is perhaps one reason for the limited success of attempts to control witches' broom. The factors involved in the production of basidiocarps, in the deposition and germination of basidiospores and in the establishment of infection need to be known to achieve a rational programme of disease control. In the present studies some of these aspects were studied in the laboratory and glasshouse under controlled conditions. The results are now discussed further in relation to the epidemiology and control of the disease.

Experiments in Section I indicated that few basidiocarps were produced on brooms kept in cabinets with a regime of 23 wet/1 h dry (Table I.2) or on those kept in the dark (Table I.8). These features might be exploited under field conditions when dead brooms are pruned. Instead of removing brooms from the cocoa plantations and burning them, with loss of nutrients, they could be placed in heaps and covered either with soil or with leaves from nearby crops, e.g. bananas. It seems likely that under these conditions few basidiocarps would develop and also other micro-organisms might colonize the brooms leading to their decomposition and the re-cycling of nutrients.

The low production of basidiocarps on brooms kept wet might account in part for the low incidence of witches' broom reported from some areas of high rainfall. For example, the disease has never been serious in the eastern valley of the Northern Range in Trinidad where the annual rainfall

is usually between 3000 and 4000 mm (Baker & Holliday, 1957). This supposes that the rate of disease development is related more to the amount of basidiospores produced within each site than to dissemination of spores from other areas. Movement of spores from a cocoa plantation is likely to be restricted by the canopy of the cocoa and shade trees. The limited studies on basidiospore dispersal suggest that they are not transported far; the maximum distance reported by Baker & Holliday (1957) was 100 m though Solorzano (1977) found that infections developed on young plants 200 m from the nearest cocoa planting with witches' broom.

The data of Table I.2, Table I.6 and Table II.2 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. However, the data of Table I.9 suggest that within local areas the production of inoculum might be influenced considerably by the type of cocoa grown. In this experiment, fewer basidiocarps developed on brooms from the ICS cultivars 1 and 95 at Pichilingne than on those from Scavina 6, Scavina 12 or UF 168. It could be argued that other factors influencing basidiocarp production on brooms were not controlled in this experiment, apart from size of broom which was kept fairly uniform. 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 Sca 6 to ICS 1 relates to increasing quantities of old, unproductive brooms in the samples, though this seems unlikely. Ideally, the productivity of brooms on the different cocoa cultivars 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 these ICS cultivars generally yield well. They

could thus be a type to exploit.

Data of Table I.10 suggest that basidiocarps develop 4 to 7½ months after the broom has formed. It is interesting to relate these data to the rainfall in different areas where cocoa is grown (Table A) to determine likely effects on the development of epidemics. In Pichilingue, for example, where generally six successive months of dry weather occur annually, there is little chance that brooms formed during the rainy season will produce basidiocarps in the same year and this would tend to favour control by removing brooms. On the other hand, in areas like Belem where no dry season occurs, there is no marked interruption in basidiocarp production. In this area the intensity of cocoa flushing is probably the most important factor and control by pruning brooms is difficult. Recent data from Benevides (near Belem) indicate that, despite pruning, inoculum within the experimental area was sufficient throughout the year to result in new infections (Andebrhan & Bastos, 1982). The absence of infected vegetative shoots during the period December to March was associated with lack of new shoots in the cocoa trees but fruits and flower cushions became infected. Probably some of these infections came from cankers on cushions, but the authors suggested that most were initiated by basidiospores from surrounding areas where there was no sanitation. The rainfall distribution in Bahia, where there is a vast area of susceptible cocoa still free of witches' broom, is similar to that of Belem and this emphasizes the potential threat should this fungus be introduced by chance to that area. In the other areas of Ouro Preto, Manaus and Trinidad, a short dry season occurs, thus indicating that the period of basidiocarp production might be shorter than in Belem though much longer than in Pichilingue.

Some aspects of the pathogenic variability of *C. perniciosa* and the reactions of cocoa cultivars to different pathotypes have been reported by

Wheeler & Mepsted (1982). The data of Table III.5 and Table III.6 confirm the differences in virulence of isolates from Brazil and Ecuador obtained by these authors and the reactions of Scavina cultivars (Fonsenca & Santos, 1982). Additionally, the present results suggest that even in one locality there are significant differences in the fungal population in that the source of inoculum influenced to some extent the severity of symptom expression on inoculated plants (Table III.5). This aspect requires further investigation in view of its importance in screening for resistance.

The other results of this investigation can be less readily related to aspects of epidemiology and control but they are of fundamental interest to the study of the fungus in infected tissues. The mechanism by which the fungus induces marked swelling of the shoots remains largely unexplored though it has been assumed to result from the production of growth substances, possibly gibberellins. As indicated earlier, the marked hypertrophy of the stem base which follows inoculation of the hypocotyl at 30° C (Fig. III.1) could provide a useful system for investigating these phenomena. Also, the estimation of fungal biomass in infected tissues by glucosamine assays might be used to determine the ability of the fungus to grow in particular cultivars and thus indicate mechanisms of resistance which depend on reducing fungal growth and limiting its potential for broom formation and basidiocarp production.

TABLE A: Rainfall averages of different cocoa growing areas (based on Wood, 1975; CEPLAC, 1979).

Months	BRAZIL				ECUADOR	TRINIDAD
	Belem[a]	Ouro Preto[b]	Manaus[c]	Urucuca[d] (Bahia)	Pichilingue[e]	St. Augustin[f]
January	355	257	272	137	500	73
February	434	295	299	137	473	45
March	457	266	366	195	436	36
April	371	184	331	192	418	55
May	302	111	270	127	136	118
June	180	34	113	161	45	264
July	187	5	85	155	47	227
August	125	51	47	115	9	236
September	147	109	85	111	18	191
October	128	194	110	127	20	164
November	127	212	164	177	27	191
December	233	225	185	151	100	145

Averages for: [a] 8 years; [b] 3 years; [c] 10 years; [d] 43 years; [e] 14 years; [f] 40 years.

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APPENDIX

TABLE 1 Production of basidiocarps on dead brooms (Text - Tables I.2, I.3, I.4).

(a) Primordia

Broom no.	Large brooms											
	Source											
	Castanhal				Manaus				Ouro Preto			
Regime: h wet/dry	1/23	8/16	16/8	23/1	1/23	8/16	16/8	23/1	1/23	8/16	16/8	23/1
1	0	29	7	0	0	8	21	0	0	4	27	0
2	0	42	1	22	0	13	6	3	0	0	1	0
3	1	1	15	7	0	24	0	21	0	2	0	1
4	0	31	2	4	0	13	0	0	0	6	17	0
5	0	25	0	11	0	0	4	0	0	7	3	0
6	2	101	3	1	0	1	6	0	0	10	23	0
7	1	16	1	1	0	80	6	0	0	13	5	0
8	0	86	11	3	0	18	6	0	0	10	0	0
9	0	3	15	1	0	7	1	1	0	2	4	0
10	0	54	17	0	2	10	17	0	0	6	7	1

TABLE 1 CONT.....

Broom no.	Small brooms											
	Source											
	Castanhal				Manaus				Ouro Preto			
Regime: h wet/dry	1/23	8/16	16/8	23/1	1/23	8/16	16/8	23/1	1/23	8/16	16/8	23/1
1	0	0	3	0	0	7	14	0	0	3	4	0
2	0	22	6	0	0	1	4	0	0	1	0	0
3	0	41	1	0	0	17	7	3	0	3	0	1
4	0	43	0	0	0	22	8	0	0	1	19	0
5	0	12	7	1	0	28	3	0	0	13	14	0
6	0	19	7	3	0	23	7	0	0	7	1	0
7	0	6	0	0	1	35	0	0	0	10	12	0
8	2	26	0	5	0	8	4	0	0	4	0	0
9	0	2	12	1	0	35	13	0	0	0	0	0
10	0	5	0	12	0	20	29	0	0	0	0	1

TABLE 1 cont.....

(b) Mature basidiocarps

Broom no.	Large brooms											
	Source											
	Castanhal				Manaus				Ouro Preto			
Regime (h wet/dry)	1/23	8/16	16/8	23/1	1/23	8/16	16/8	23/1	1/23	8/16	16/8	23/1
1	0	21	8	0	0	6	12	0	0	2	27	0
2	0	36	1	8	0	18	6	3	0	0	0	0
3	0	1	12	5	0	21	0	21	0	2	0	0
4	0	17	0	1	0	12	0	0	0	2	17	0
5	0	18	0	6	0	0	4	0	0	4	2	0
6	0	60	1	1	0	0	2	0	0	4	14	0
7	0	15	1	0	0	36	5	0	0	8	5	1
8	0	39	4	0	0	15	3	0	0	10	0	0
9	0	0	8	0	0	6	1	1	0	2	1	0
10	0	21	8	0	0	8	12	0	0	4	7	0

TABLE 1 cont.....

Broom no.	Small brooms											
	Source											
	Castanhal				Manaus				Ouro Preto			
Regime (h wet/dry)	1/23	8/16	16/8	23/1	1/23	8/16	16/8	23/1	1/23	8/16	16/8	23/1
1	0	0	2	0	0	4	13	0	0	2	2	0
2	0	14	5	0	0	1	4	0	0	0	0	0
3	0	9	1	0	0	8	6	2	0	2	0	1
4	0	32	0	0	0	14	5	0	0	1	12	0
5	0	6	7	1	0	17	3	0	0	9	10	0
6	0	16	5	3	0	16	7	0	0	5	1	0
7	0	6	0	0	0	27	0	0	0	7	10	0
8	0	15	0	1	0	8	4	0	0	3	0	0
9	0	0	10	0	0	19	11	0	0	0	0	0
10	0	4	0	10	0	20	23	0	0	0	0	0

TABLE 2 Effect of temperature on basidiocarp production (Text - Table I.6).

(a) Primordia

Broom No.	Temperature ° C 20-25				25-30				30-35			
	3[a]	4	5	6	3	4	5	6	3	4	5	6
1	0	2	1	3	2	3	4	0	0	0	0	0
2	0	3	2	0	1	1	2	1	0	0	0	0
3	0	0	1	9	3	3	3	0	0	0	0	0
4	0	5	8	1	0	1	1	0	0	0	0	0
5	0	4	2	0	1	2	1	0	0	0	0	0
6	0	3	3	0	0	2	0	0	0	0	0	0
7	0	1	2	0	0	0	1	0	0	0	0	0
8	2	18	5	2	2	0	0	0	0	0	0	0
9	1	4	3	7	0	2	1	0	0	0	0	0
10	0	2	2	0	1	5	0	0	0	0	0	0

a 4 - week periods

TABLE 2 cont.....

(b) Mature basidiocarps

Broom No.	Temperature ° C											
	20-25				25-30				30-35			
	3[a]	4	5	6	3	4	5	6	3	4	5	6
1	0	1	1	3	0	2	3	0	0	0	0	0
2	0	2	2	0	0	0	1	1	0	0	0	0
3	0	0	1	5	0	1	2	0	0	0	0	0
4	0	4	7	0	0	1	1	0	0	0	0	0
5	0	3	2	0	0	0	1	0	0	0	0	0
6	0	3	3	0	0	2	0	0	0	0	0	0
7	0	1	2	0	0	1	0	0	0	0	0	0
8	1	17	5	0	0	0	0	0	0	0	0	0
9	0	1	3	5	0	1	1	0	0	0	0	0
10	0	1	2	0	0	5	0	0	0	0	0	0

[a] 4 - week periods

TABLE 3 The effect of temperature on the morphology of basidiocarps (Text - Table I.7).

Basidiocarp No.	Temperature ° C			
	20 - 25		25 - 30	
	Pileus diameter (mm)	Stipe length (mm)	Pileus diameter (mm)	Stipe length (mm)
1	21.5	9.5	7.0	11.0
2	19.0	8.0	8.0	12.0
3	19.5	11.0	8.5	12.0
4	8.0	8.0	8.0	11.0
5	11.0	8.0	7.0	7.0
6	11.0	8.5	9.0	11.0
7	11.0	9.0	6.0	8.0
8	4.0	6.0	9.0	11.0
9	10.0	9.0	12.0	12.0
10	6.0	5.0	9.0	12.0
11	12.0	9.0	8.0	10.0
12	6.0	6.0	8.5	9.0
13	7.0	5.0	7.0	11.0
14	5.0	8.0	7.0	11.0
15	6.0	7.0	7.0	12.0
16	12.0	8.0	8.0	11.0
17	11.0	6.0	8.0	12.0
18	10.0	8.0	7.0	11.0
19	11.0	8.0	7.0	11.0
20	11.0	7.0	8.0	12.0

TABLE 4 Effect of light intensity on the production of basidiocarps (Text - Table I.8)

(a) Primordia

Broom No.	μ E/m²/s								Darkness			
	100				10							
	3[a]	4	5	6	3	4	5	6	3	4	5	6
1	0	0	0	0	0	0	3	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	1	0	0	0	0
5	0	0	0	1	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	4	4	4	0	0	0	3	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	6	10	0	0	0	1	0	0	0	0	0
10	0	2	2	0	0	0	0	0	0	0	0	0
11	0	0	5	1	0	1	0	0	0	0	3	0
12	0	1	2	0	0	0	2	0	0	0	0	0
13	0	11	6	3	0	2	0	0	0	0	2	0
14	2	5	19	1	0	0	1	0	0	0	0	0
15	2	8	10	0	0	0	11	5	0	0	0	0
16	0	7	6	2	0	16	0	0	0	0	0	0
17	0	0	7	2	0	0	1	0	0	0	0	0
18	0	0	8	1	0	2	0	0	0	0	0	0

TABLE 4 cont.....

Broom No.	$\mu E/m^2/s$ 100				10				Darkness			
	3[a]	4	5	6	3	4	5	6	3	4	5	6
19	0	1	4	3	0	0	3	0	0	0	0	0
20	1	3	11	0	0	0	0	2	0	0	0	0

[a] 4 - week periods

TABLE 4 cont.....

(b) Mature basidiocarps

Broom No.	μE/m²/s 100				10				Darkness			
	3[a]	4	5	6	3	4	5	6	3	4	5	6
1	0	0	0	0	0	0	2	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	1	0	0	0	0
5	0	0	0	1	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	4	3	4	0	0	0	2	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	4	6	0	0	0	1	0	0	0	0	0
10	0	1	1	0	0	0	0	0	0	0	0	0
11	0	0	5	1	0	0	0	0	0	0	1	0
12	0	0	1	0	0	0	2	0	0	0	0	0
13	0	6	4	2	0	2	0	0	0	0	0	0
14	2	3	17	0	0	0	0	0	0	0	0	0
15	0	8	4	0	0	0	11	0	0	0	0	0
16	0	4	3	2	0	5	0	0	0	0	0	0
17	0	0	2	2	0	0	0	0	0	0	0	0
18	0	0	7	1	0	1	0	0	0	0	0	0

TABLE 4 cont.....

(b) Mature basidocarps

Broom No.	μE/m²/s 100				10				Darkness			
	3[a]	4	5	6	3	4	5	6	3	4	5	6
19	0	1	4	3	0	0	0	0	0	0	0	0
20	1	0	6	0	0	0	0	0	0	0	0	0

[a] 4 - week periods

TABLE 5 Total number of basidiocarps produced on brooms of different cocoa cultivars (Text - Table I.9).

(a) Primordia:

Broom No.	Cultivars									
	Sca 6	UF 168	Sca 12	IMC 67	Catongo	EET 400	UF 677	ICS 39	ICS 95	ICS 1
1	17	48	10	12	9	1	19	7	14	13
2	30	1	28	9	23	12	19	17	0	3
3	41	32	16	16	8	9	8	21	11	1
4	13	0	21	14	35	6	0	4	20	19
5	19	21	28	6	21	5	0	7	2	7
6	34	33	1	5	4	20	15	9	0	3
7	23	13	24	26	16	29	4	7	6	2
8	7	0	14	13	6	19	1	18	13	14
9	57	0	32	20	4	11	4	4	1	3
10	23	0	5	13	6	9	8	8	10	0

TABLE 5 cont.....

(b) Mature basidiocarps:

Broom No.	Cultivars									
	Sca 6	UF 168	Sca 12	IMC 67	Catongo	EET 400	UF 677	ICS 39	ICS 95	ICS 1
1	7	39	10	10	2	1	10	3	9	7
2	22	1	24	2	11	11	14	15	0	3
3	34	9	13	9	4	3	8	18	11	1
4	12	0	8	12	25	5	0	2	10	16
5	19	17	22	5	18	3	0	4	1	7
6	29	29	0	4	3	10	13	0	0	3
7	20	9	22	23	13	23	4	2	4	1
8	2	0	3	12	3	12	1	18	8	7
9	41	0	19	14	1	9	4	2	1	3
10	23	0	3	8	6	2	7	8	4	0

TABLE 6 Primordia and mature basidiocarps produced on brooms of different ages (Text - Table I.10)

Broom formed (1981)		Broom no. 1	2	3	4	5	6	7	8	9	10
27 March:	Primordia	44	42	15	19	28	0	29	24	59	19
	Mature	30	42	10	9	25	0	24	9	53	15
28 April:	Primordia	37	40	34	16	19	10	41	20	3	33
	Mature	33	38	27	15	8	9	34	18	2	31
28 May:	Primordia	22	51	10	5	34	10	23	72	15	49
	Mature	11	36	9	5	21	7	10	57	14	38
30 June:	Primordia	1	0	10	12	44	30	25	34	32	20
	Mature	1	0	5	10	28	24	23	27	27	20

TABLE 7 Basidiocarps produced on brooms from Pichilingue at nodes and internodes with or without wounding. (Text - Table I.11)

Broom no.	Site of basidiocarp	Treatment: None							Wounding at node							Wounding at internode						
		3[a]	4	5	6	7	8	9	3	4	5	6	7	8	9	3	4	5	6	7	8	9
1	Node	0	0	0	1	0	0	0	0	0	0	1	3	0	0	1	4	0	4	4	0	1
	Internode	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
2	Node	0	0	0	2	6	1	4	0	36	2	0	21	5	5	0	3	0	0	0	2	1
	Internode	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0
3	Node	0	26	0	0	4	1	1	7	18	0	1	15	2	0	7	9	0	0	19	2	0
	Internode	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	3	0	0	1	0	0
4	Node	20	22	0	0	0	0	0	0	0	0	0	5	0	0	1	20	0	1	2	0	0
	Internode	1	5	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	1	0	0	0
5	Node	0	0	0	0	1	0	0	0	2	1	0	0	0	1	0	12	1	2	0	1	0
	Internode	0	0	0	0	0	0	0	5	2	0	0	4	0	0	0	1	0	0	0	0	0
6	Node	0	0	0	0	11	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
	Internode	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
7	Node	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	3	1	4	8	0	3
	Internode	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0

[a] 4 - week periods

TABLE 8 Weekly production of basidiocarps on brooms from three regions of Brazil kept in four different regimes of wet/dry periods. (Text - Fig I.3).

Regime (h wet/dry)	1/23 (A)			8/16 (B)			16/8 (C)			23/1 (D)		
Week no.	O.Preto	Castanhal	Manaus	O.Preto	Castanhal	Manaus	O.Preto	Castanhal	Manaus	O.Preto	Castanhal	Manaus
1	0	5	1	3	3	4	12	3	3	1	15	1
2	0	0	0	2	0	11	27	2	8	0	5	12
3	0	0	0	5	18	22	4	18	48	2	24	15
4	0	1	1	9	41	75	3	16	19	0	2	0
5	0	0	1	11	139	40	3	9	5	1	4	0
6	0	0	0	0	3	1	4	13	2	0	16	0
7	0	0	0	4	43	27	2	15	9	0	0	0
8	0	0	0	8	70	24	5	0	3	0	0	0
9	0	0	0	16	86	37	9	10	5	0	0	0
10	0	0	0	10	3	10	14	5	4	0	6	0
11	0	0	0	5	43	4	26	11	13	0	0	0
12	0	0	0	3	24	12	6	3	16	0	0	0
13	0	0	0	3	13	5	9	1	9	0	0	0
14	0	0	0	15	82	86	3	0	3	0	0	0
15	0	0	0	6	6	4	10	2	9	0	0	0

TABLE 9 Weekly production of basidiocarps on brooms transferred to regime B after 15 weeks in regimes A, C and D (Text - Fig I.6).

Broom no.	Week no. 16			17			18			19		
Regime	A	C	D	A	C	D	A	C	D	A	C	D
1	6	0	0	0	12	0	2	2	0	0	0	0
2	1	1	0	2	0	0	0	0	0	0	0	0
3	1	0	0	0	34	0	0	0	0	0	0	0
4	3	2	0	2	0	0	1	0	0	0	0	0
5	0	0	0	2	1	0	1	0	0	2	0	0
6	0	0	0	3	6	0	0	0	0	0	2	2
7	0	1	0	0	3	0	0	0	0	0	0	0
8	1	1	0	2	5	0	0	0	0	0	0	0
9	0	2	0	0	1	0	0	0	0	0	1	1
10	0	1	0	0	0	0	0	0	0	1	0	0

TABLE 10 Water content of brooms subjected to different regimes of wet/dry periods (Text - Fig I.7).

Regime (h wet/dry)	A 1/23	Sample 1	2	3	4
1 h Wetting	(a)	2.2	2.1	2.1	2.1
	(b)	1.4	1.4	1.5	1.6
3 h Drying	(a)	1.4	1.3	1.2	1.3
	(b)	1.15	1.1	1.0	0.7
6 h Drying	(a)	1.5	1.4	1.2	1.3
	(b)	1.2	1.1	0.95	1.0
9 h Drying	(a)	1.7	1.6	1.8	1.9
	(b)	1.25	1.35	1.5	1.5
12 h Drying	(a)	1.6	2.0	1.9	1.7
	(b)	1.3	1.55	1.55	1.4
15 h Drying	(a)	1.1	1.4	1.4	1.3
	(b)	0.85	1.2	1.15	1.2
18 h Drying	(a)	1.4	1.4	1.4	1.6
	(b)	1.2	1.15	1.2	1.35
21 h Drying	(a)	1.3	1.25	1.5	1.8
	(b)	1.0	1.15	1.1	1.7

(a): wet weight; (b): dry weight (g).

TABLE 10 cont.....

Regime (h wet/dry)	B 8/16	Sample 1	2	3	4
3 h Wetting	(a)	2.9	2.9	2.6	2.5
	(b)	1.6	1.55	1.4	1.5
6 h Wetting	(a)	1.9	1.9	2.0	2.0
	(b)	0.75	0.85	1.0	0.9
3 h Drying	(a)	2.9	2.7	2.1	1.9
	(b)	1.25	1.45	1.15	1.0
6 h Drying	(a)	1.4	1.3	1.1	1.2
	(b)	0.8	0.8	0.8	0.8
9 h Drying	(a)	1.3	1.3	1.4	1.5
	(b)	1.0	0.8	0.8	1.0
12 h Drying	(a)	1.5	1.1	1.5	1.1
	(b)	1.0	0.8	1.1	1.0
15 h Drying	(a)	1.2	1.2	1.4	1.3
	(b)	1.0	1.1	1.1	1.1

(a): wet weight; (b): dry weight (g).

TABLE 10 cont.....

Regime C (h wet/dry) 16/8		Sample 1	2	3	4
3 h Wetting	(a)	2.3	3.7	2.0	1.6
	(b)	1.2	2.1	1.0	1.05
6 h Wetting	(a)	1.4	1.7	1.9	2.0
	(b)	0.6	0.6	0.7	0.75
9 h Wetting	(a)	3.4	2.3	2.9	2.3
	(b)	1.55	1.15	1.4	1.05
12 h Wetting	(a)	3.0	3.3	2.1	1.9
	(b)	1.5	1.55	1.0	1.0
15 h Wetting	(a)	2.4	2.0	2.1	1.8
	(b)	1.2	1.0	1.0	1.0
3 h Drying	(a)	1.8	1.8	1.5	1.4
	(b)	0.9	1.1	1.1	0.95
6 h Drying	(a)	1.3	2.1	1.5	1.6
	(b)	0.8	1.3	1.0	1.1

(a): wet weight; (b): dry weight (g).

TABLE 10 cont.....

Regime D (h wet/dry) 23/1		Sample 1	2	3	4
3 h Wetting	(a)	2.7	2.6	1.6	1.7
	(b)	0.65	0.55	0.35	0.35
6 h Wetting	(a)	1.9	2.7	2.4	2.4
	(b)	0.45	0.6	0.5	0.5
9 h Wetting	(a)	2.0	3.1	2.3	2.2
	(b)	0.45	0.6	0.5	0.5
12 h Wetting	(a)	1.5	2.0	2.4	2.0
	(b)	0.3	0.4	0.6	0.5
15 h Wetting	(a)	1.8	1.9	1.9	2.0
	(b)	0.4	0.4	0.45	0.5
18 h Wetting	(a)	2.7	3.0	2.1	2.5
	(b)	0.7	0.8	0.5	0.45
21 h Wetting	(a)	1.25	1.4	1.3	1.6
	(b)	0.3	0.35	0.3	0.4
1 h Drying	(a)	1.3	1.7	2.3	2.3
	(b)	0.3	0.4	0.7	0.7

(a): wet weight; (b): dry weight (g).

TABLE 11 Solubility of broom wood in 1% NaOH (%) (Text - Fig I.8).

Regime (h wet/dry)	Source of broom: Castanhal		Manaus		Ouro Preto	
	Sample 1	2	1	2	1	2
Untreated brooms	54.4	54.0	52.4	52.7	52.5	53.6
1/23 (A)	53.2	53.5	56.8	56.7	52.1	53.6
8/16 (B)	58.9	59.8	59.1	56.4	56.8	58.0
16/8 (C)	58.5	58.0	55.4	55.5	55.4	56.7
23/1 (D)	54.4	52.8	60.2	55.0	52.8	55.5

TABLE 12 Stem swelling induced on cocoa using basidiospores from basidiocarps produced under different temperature and light regimes. (Text - Table II.1).

Plant no.	Conditions on which basidiocarps were induced				Uninoculated control
	Temperature (°C)		Light intensity ($\mu E/m^2/s$)		
	20-25	25-30	10	100	
1	0.66[a]	1.10	0.50	0.82	0.41
2	0.69	0.46	0.76	0.68	0.47
3	0.74	1.00	0.69	1.03	0.47
4	0.64	0.70	0.69	0.60	0.41
5	0.67	0.83	0.66	0.78	0.40

[a] Stem diameter (cm), 8 weeks after inoculation.

TABLE 13 Basidiospores discharged (thousands) under different temperature, relative humidity and light (Text - Table II.2, Fig II.1).

Light

r.h. %		80			85			90			95			100		
Temp (°C)	Repl.	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
10		4	8	28	16	8	12	4	28	12	8	4	8	20	4	28
15		160	124	184	200	300	288	200	268	184	208	204	120	200	304	172
20		348	352	352	420	564	444	424	528	340	356	364	324	400	296	416
25		620	500	632	316	684	604	436	448	452	404	380	344	384	324	204
30		52	68	48	64	56	68	128	80	132	4	24	4	140	228	132

Dark

r.h. %		80			85			90			95			100		
Temp (°C)	Repl.	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
10		4	8	20	12	4	8	4	28	8	4	4	4	12	8	28
15		192	164	144	220	348	276	232	220	268	188	168	156	156	244	152
20		320	364	312	392	484	396	316	608	388	352	316	360	388	276	440
25		520	460	468	388	580	472	368	392	404	428	348	320	340	312	248
30		40	64	40	72	80	60	116	64	100	48	40	28	148	48	104

FIGURE 1: Regressions of basidiospore on temperature at five relative humidities (a), 80%; (b), 85%; (c), 90%; (d), 95%; (e), 100%.

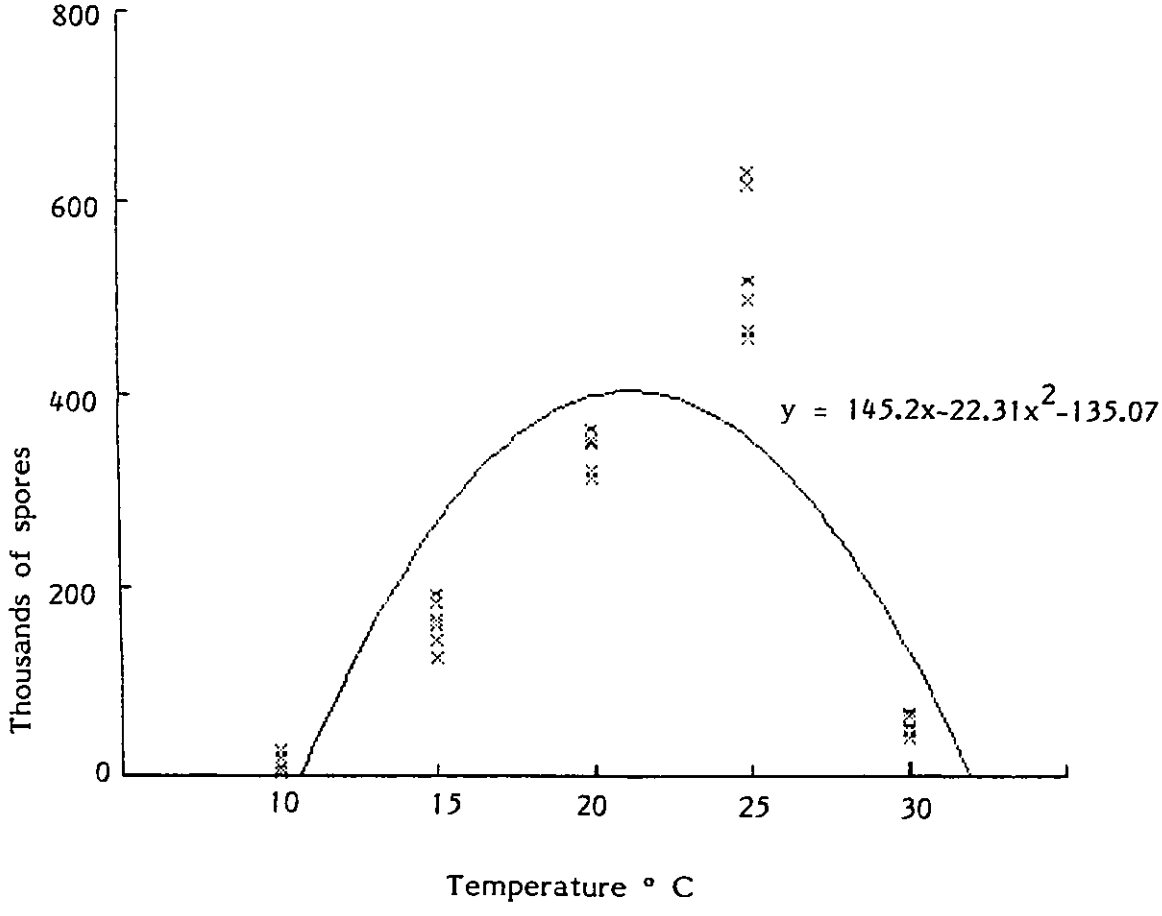
800
600
400
200
0
Thousands of spores
y = 145.2x-22.31x2-135.07
10
15
20
25
30
Temperature ° C

FIGURE 1 (a)

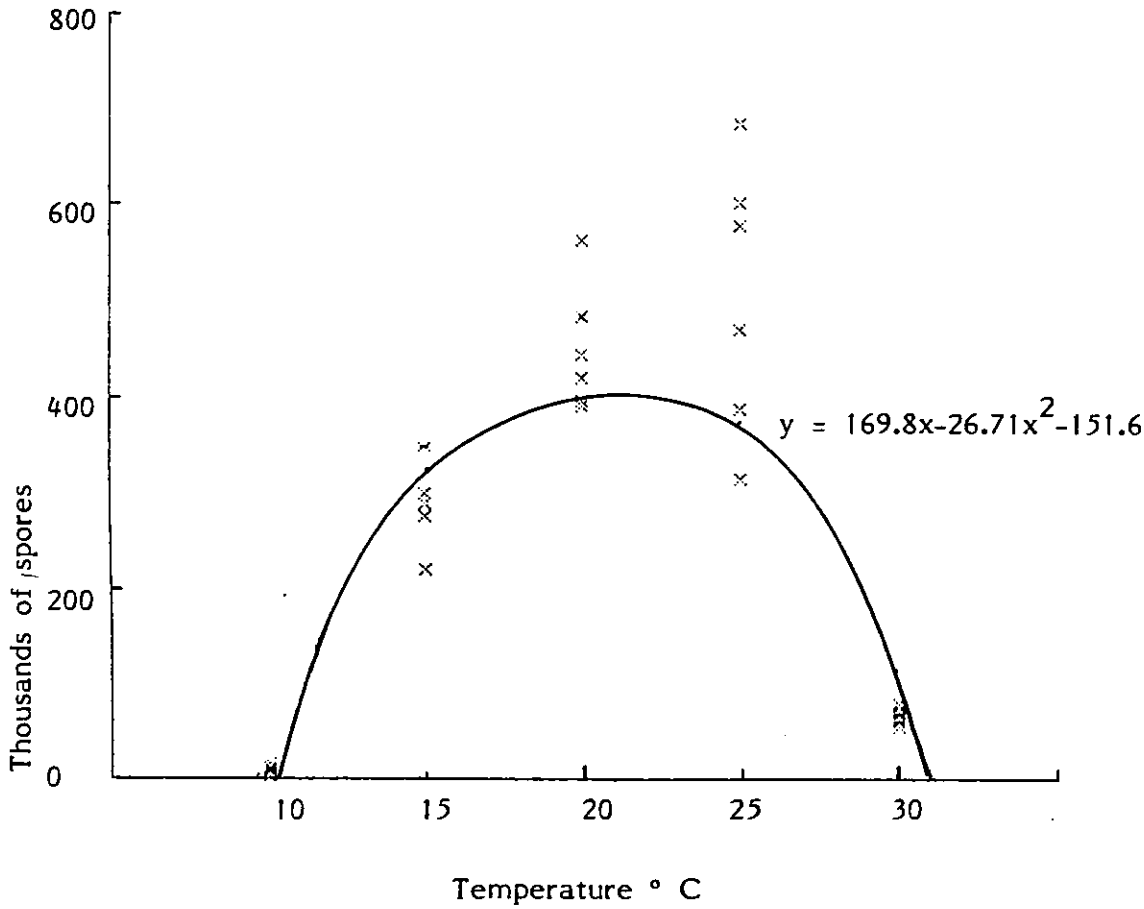
800
600
400
200
0
Thousands of spores
10
15
20
25
30
Temperature ° C
y = 169.8x-26.71x2-151.6

FIGURE 1 (b)

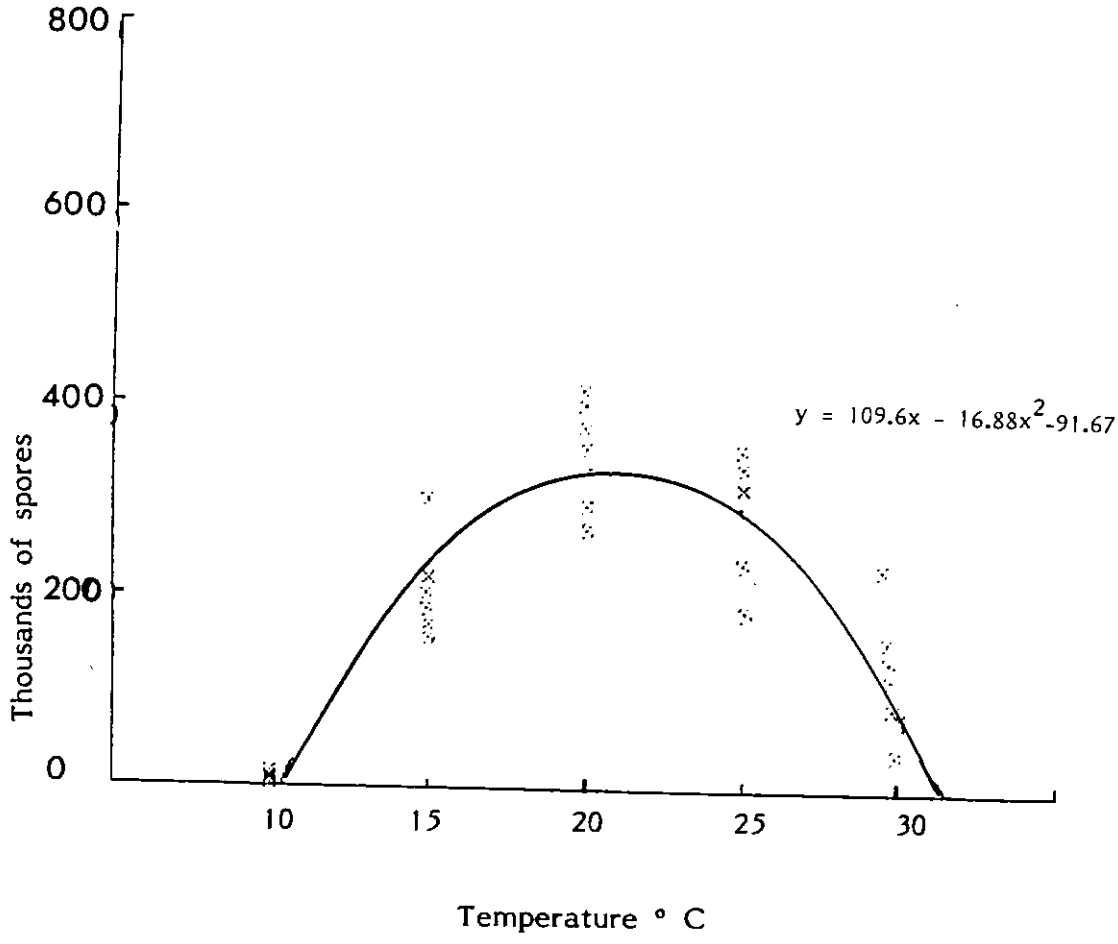
800
600
400
200
0
Thousands of spores
y = 109.6x - 16.88x^2-91.67
10
15
20
25
30
Temperature ° C

FIGURE 1 (e)

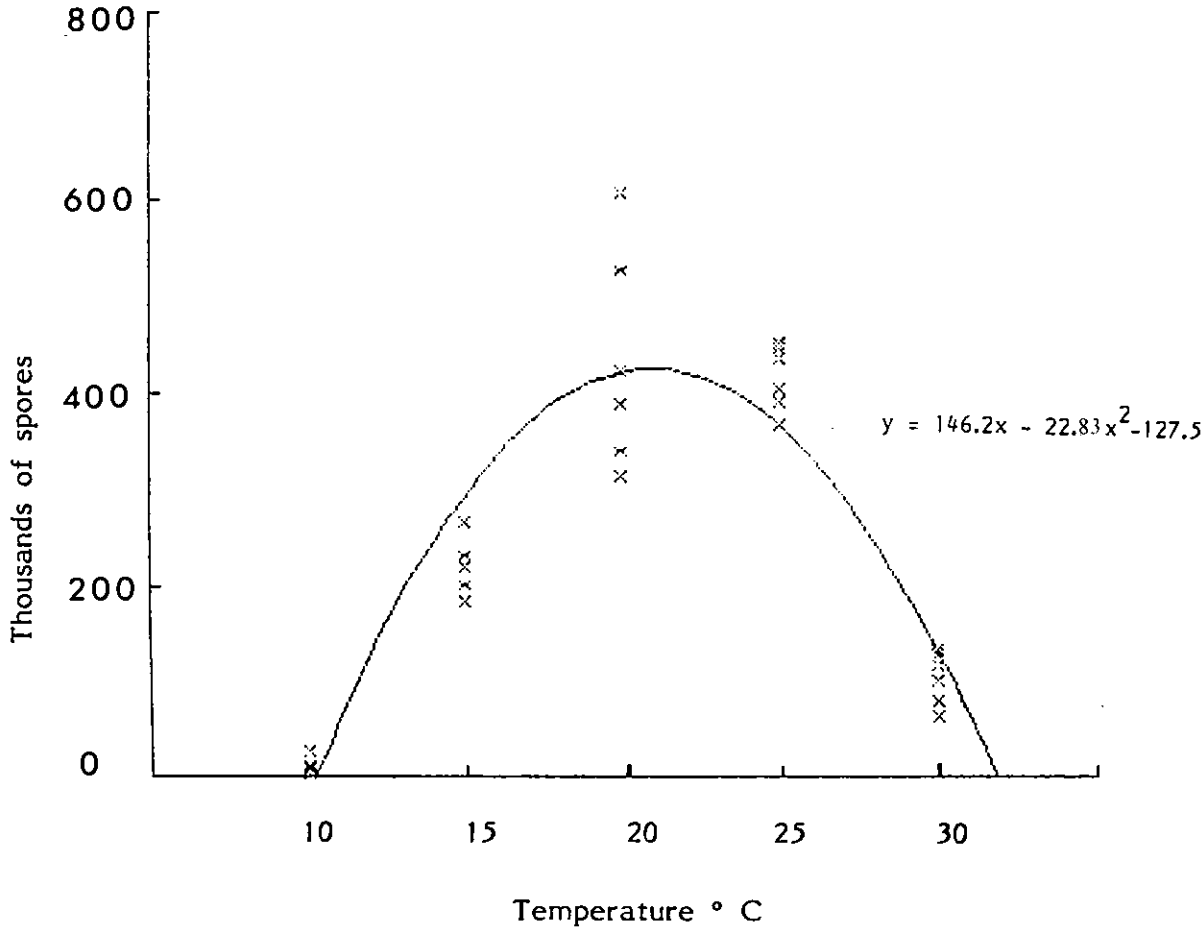
800
600
400
200
0
Thousands of spores
y = 146.2x - 22.83x2-127.5
10
15
20
25
30
Temperature ° C

FIGURE 1 (c)

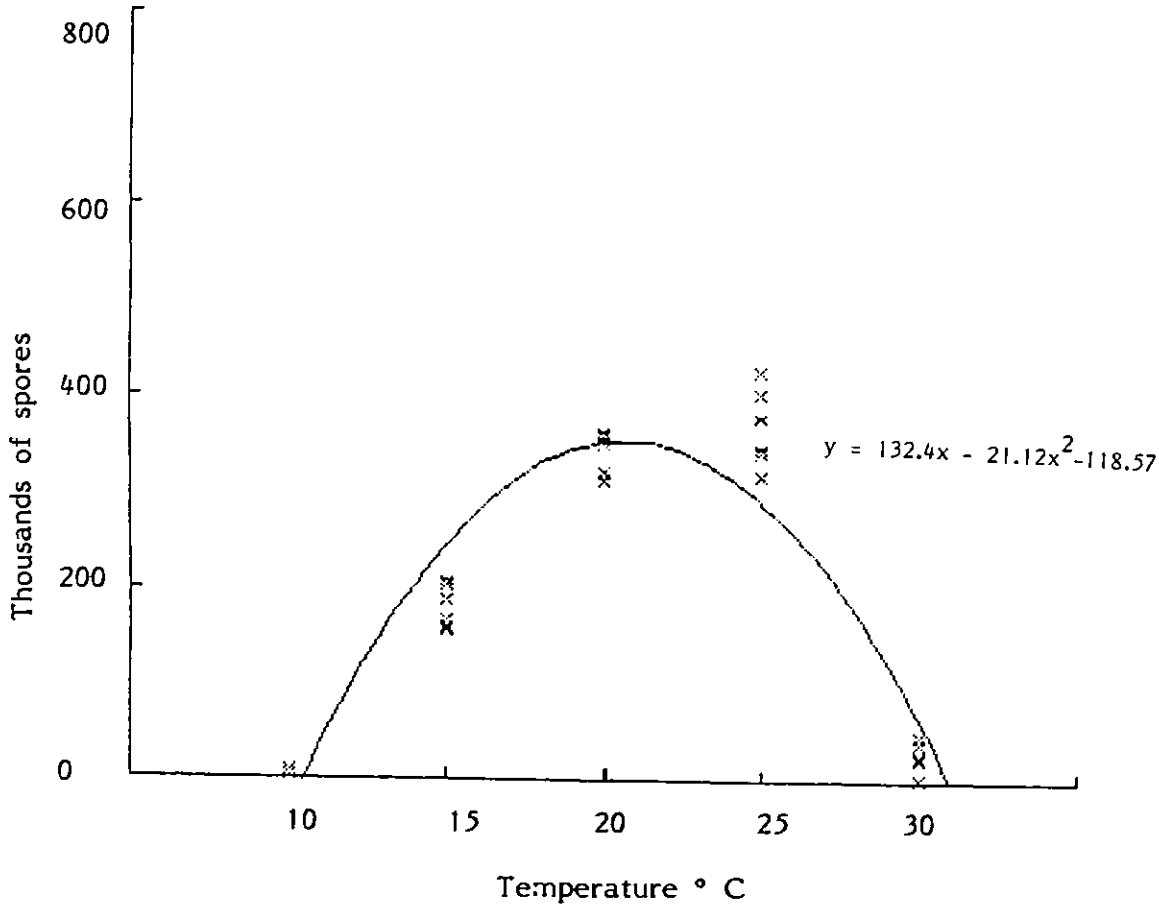
800
600
400
200
0
Thousands of spores
y = 132.4x - 21.12x^2-118.57
10
15
20
25
30
Temperature ° C

FIGURE 1 (d)

TABLE 14 Effect of temperature and light on the germination (ARCSIN $\sqrt{\%}$) of basidiospores and germ tube length (μm) . (Text - Table II.4).

a) Germination

Sample	15°C		20°C		25°C		30°C	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
1	19.73	20.09	79.86	57.42	90.00	90.00	36.81	65.20
2	18.44	7.49	90.00	75.94	70.54	90.00	53.19	50.24
3	26.56	11.83	73.46	63.44	78.46	90.00	53.97	53.85
4	16.43	16.43	75.00	79.53	90.00	90.00	50.42	52.30
5	20.70	24.88	71.56	73.89	79.53	90.00	60.00	62.94
6	16.00	18.72	71.56	90.00	90.00	90.00	65.57	64.97
7	21.22	16.74	78.17	79.53	90.00	90.00	65.65	58.69
8	9.28	14.06	80.72	81.28	90.00	90.00	70.18	59.15
9	17.26	15.45	90.00	66.42	90.00	90.00	65.50	63.44
10	19.37	13.05	90.00	80.19	90.00	90.00	59.34	59.74
11	12.66	18.15	70.54	63.94	75.70	90.00	50.07	57.17
12	17.76	15.45	60.00	60.87	90.00	90.00	57.48	62.31
13	16.32	15.00	52.71	71.56	90.00	90.00	59.28	56.79
14	9.63	23.11	61.89	69.64	81.87	90.00	59.15	48.22
15	17.85	10.94	66.50	62.24	90.00	90.00	56.79	65.57

TABLE 14 cont.....

b) Germ tube length (μm)[a]

Sample	15°C		20°C		25°C		30°C	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
1	5.00	6.25	18.75	10.00	67.50	80.00	77.50	57.50
2	6.25	3.75	27.50	11.25	65.00	92.50	42.50	62.50
3	5.00	2.50	45.00	7.50	82.50	92.50	55.00	87.50
4	7.50	5.00	21.25	20.00	70.00	72.50	55.00	90.00
5	3.75	6.25	30.00	22.50	112.50	57.50	72.50	62.50
6	3.75	6.25	22.50	37.50	85.00	55.00	72.50	75.00
7	7.50	7.50	22.50	10.00	65.00	70.00	80.00	62.50
8	2.50	2.50	32.50	22.50	90.00	70.00	72.50	90.00
9	3.75	3.75	40.00	23.75	75.00	105.00	72.50	75.00
10	5.00	5.00	50.00	35.00	87.50	105.00	67.50	75.00
11	5.00	8.75	42.50	42.50	75.00	72.50	60.00	65.00
12	2.50	7.50	20.00	37.50	110.00	107.50	85.00	67.50
13	3.75	5.00	32.50	20.00	87.50	87.50	52.50	37.50
14	5.00	6.25	27.50	47.50	92.50	65.00	62.50	77.50
15	2.50	3.75	47.50	25.00	72.50	87.50	82.50	70.00
16	3.75	2.50	22.50	47.50	92.50	87.50	87.50	52.50
17	6.25	2.50	20.00	12.50	57.50	62.50	70.00	82.50
18	2.50	5.00	20.00	12.50	55.00	77.50	40.00	40.00
19	8.00	6.25	40.00	48.75	62.50	80.00	67.50	47.50
20	5.00	5.00	10.00	7.50	80.00	77.50	82.50	72.50

[a] 3 h after deposition on water agar film.

TABLE 15 Maximum diameter (cm) of inoculated shoots, relative to the corresponding controls, on three cocoa cultivars inoculated with Pichilingue isolate of *C. perniciosa* (Text - Table III.5)

Cultivars		Sca 6			EET 400			UF 168		
Inoculum Source[a]		A	B	C	A	B	C	A	B	C
Plant no.	1	0.20	0.20	0.20	0.60	0.30	0.50	0.60	0.20	0.50
	2	0.25	0.25	0.35	0.10	0.60	0.20	0.30	0.20	0.20
	3	0.40	0.20	0.30	0.25	0.50	0.45	0.35	0.15	0.35
	4	0.25	0.15	0.35	0.20	0.25	0.10	0.15	0.12	0.25
	5	0.20	0.20	0.30	0.20	0.65	0.25	0.30	0.15	0.50
	6	0.25	0.25	0.25	0.25	0.35	0.40	0.10	0.10	0.25
	7	0.20	0.10	0.40	0.40	0.50	0.35	0.50	0.20	0.45
	8	0.35	0.15	0.25	0.10		0.25	0.35		0.35
	9	0.20	0.20	0.30	0.50		0.35			
	10	0.30	0.10	0.20	0.40		0.40			
	11	0.20								
	12	0.30								

[a] Basidiospores collected from basidiocarps produced on brooms of :

(A) Sca 6; (B) EET 400 and (C) UF 168.

TABLE 16 Increase in main shoot diameter (cm) over controls of cocoa seedlings inoculated with Brazilian (O. Preto) isolate of *C. perniciosa* (Text - Table III.6).

Catongo				IMC 67				Sca 6		Sca 12	
Pl.no.	Increase diam.	Pl.no.	Increase diam.	Pl.no.	Increase diam.	Pl.no.	Increase diam.	Pl.no.	Increase diam.	Pl.no.	Increase diam
1	0.16	24	0.09	1	0.28	20	0.11	1	0.09	1	0.01
2	0.25	25	0.16	2	0.28	21	0.25	2	0.04	2	0.16
3	0.20	26	0.18	3	0.18	22	0.40	3	0.07	3	0.01
4	0.22	27	0.16	4	0.05	23	0.17	4	0.02	4	0.18
5	0.12	28	0.17	5	0.21	24	0.14	5	0.16	5	0.10
6	0.17	29	0.18	6	0.25	25	0.29	6	0.14	6	0.07
7	0.21	30	0.18	7	0.21	26	0.30	7	0.26	7	0.12
8	0.17	31	0.18	8	0.20	27	0.28	8	0.24	8	0.05
9	0.29	32	0.29	9	0.07	28	0.04	9	0.13	9	0.06
10	0.24	33	0.26	10	0.23	29	0.25	10	0.07	10	0.10
11	0.14	34	0.21	11	0.27	30	0.23	11	0.16	11	0.08
12	0.24	35	0.21	12	0.24	31	0.14	12	0.08	12	0.12
13	0.20	36	0.16	13	0.14	32	0.27	13	0.14	13	0.18
14	0.09	37	0.21	14	0.09	33	0.07	14	0.08	14	0.19
15	0.09	38	0.14	15	0.25	34	0.20	15	0.09	15	0.12
16	0.05	39	0.20	16	0.25	35	0.26	16	0.16	16	0.22
17	0.06	40	0.20	17	0.19	36	0.27	17	0.08	17	0.15
18	0.20	41	0.10	18	0.19	37	0.09	18	0.12	18	0.06
19	0.24	42	0.15	19	0.10			19	0.09	19	0.18
20	0.15	43	0.23					20	0.06		
21	0.08	44	0.15								
22	0.14	45	0.24								
23	0.23	46	0.18								

TABLE 17 Effect of temperature on stem base swelling (cm) of Nacional x Sca 12 seedlings, 4 weeks after inoculation of hypocotyl with an isolate of *C. perniciosa* from Pichilingue, Ecuador. (Text - Fig III.5).

	Temperature ° C							
	15		20		25		30	
Plant No.	Inoc.	Control	Inoc.	Control	Inoc.	Control	Inoc.	Control
1	0.30	0.30	0.45	0.38	0.80	0.40	1.60	0.36
2	0.40	0.40	0.80	0.40	0.75	0.35	0.88	0.42
3	0.39	0.34	0.40	0.34	0.53	0.50	1.10	0.46
4	0.44	0.34	1.18	0.43	0.78	0.46	0.95	0.46
5	0.30	0.30	0.78	0.43	0.98	0.40	0.99	0.40
6	0.35	0.34	0.40	0.35	0.99	0.52	1.40	0.40
7	0.40	0.40	0.89	0.43	0.78	0.38	1.20	0.40
8	0.40	0.33	0.50	0.40	0.80	0.40	1.40	0.46
9	0.38	0.38	0.60	0.43	0.65	0.45	0.99	0.36
10	0.50	0.37	0.63	0.38	0.80	0.47	1.19	0.50
11	0.40	0.34	0.65	0.40	1.00	0.50	1.58	0.55
12	0.43	0.30	0.60	0.36	1.30	0.35	1.43	0.40
13	0.34	0.30	0.60	0.40	1.09	0.45	0.99	0.50
14	0.45	0.40	0.62	0.40	0.70	0.46	1.20	0.44
15	0.39	0.36	0.62	0.40	0.85	0.45	1.43	0.40

TABLE 18 Mycelium dry weight and corresponding glucosamine content of four isolates of *C. perniciosa* (Text - Table IV.I, Fig IV.1).

Sampling Time (days)	Isolate	Mycelium dry wt (mg)	Corresponding glucosamine Total (μg)	C.F.(μg/mg)
15	Chigorodo	64.5	520	8.06
		59.4	520	8.76
		57.1	507	8.88
	Pichilingue	41.0	440	10.73
		55.4	453	8.17
		60.0	453	7.55
	Trinidad	51.0	413	8.10
		51.4	360	7.00
		53.0	587	11.07
	Ouro Preto	53.0	307	5.79
		50.4	453	8.98
		49.5	653	13.20
21	Chigorodo	122.7	1005	8.19
		120.1	724	6.03
		114.6	1207	10.53
	Pichilingue	107.6	1076	10.00
		162.2	1127	6.95
		135.3	1127	8.33
	Trinidad	170.7	1219	7.04
		107.6	1218	11.32
		146.5	1219	8.32
	Ouro Preto	183.3	1351	7.37
		146.2	1247	8.53
		123.1	1454	11.81
28	Chigorodo	149.1	950	6.37
		153.8	1200	7.80
		169.5	1200	7.08
	Pichilingue	198.9	1400	7.04
		129.9	1200	9.24
		128.3	1200	9.35

TABLE 18 cont.....

Sampling Time (days)	Isolate	Mycelium dry wt (mg)	Corresponding glucosamine Total (µg)	C.F. (µg/mg)
28	Trinidad	201.0	1600	7.96
		204.9	1500	7.32
		195.8	1600	8.17
	Ouro Preto	181.5	1800	9.92
		233.9	1600	6.84
		207.9	1800	8.66

TABLE 19 Glucosamine recovered from Dowex 50 (200-400 mesh) columns with 20 ml 2 N HCl as effluent liquid[a]. Text - Table IV.2

Fraction (2 ml) eluted	Glucosamine (μg)	
	1	2
1 st	0.0	0.0
2 nd	1.7	1.7
3 rd	36.9	36.7
4 th	43.7	44.1
5 th	17.5	17.5
6 th	7.0	7.0
7 th	1.8	1.8
8 th	0.0	0.0

[a] A total of 100 μg glucosamine hydrochloride in 10 ml distilled water was pipetted onto each column.

TABLE **20** Amounts of mycelium (μg dry weight/mg dried broom) as determined by glucosamine assays in brooms induced by *C. perniciosa* from Pichilingue, Ecuador. (Text - Table IV.3).

Region of broom	Replicate	Green brooms Na 32 x UIT1		Dying brooms Na 32 x UIT1		Dead brooms IMC 67	
		Cortex	Wood	Cortex	Wood	Cortex	Wood
Node	1	66.2	110.3	195.3	183.1	410.8	326.3
	2	44.1	157.2	195.3	190.1	409.9	293.0
	3	51.6	110.3	239.4	175.6	392.5	292.0
Internode	1	58.6	81.2	183.1	133.8	368.1	227.7
	2	44.1	81.2	183.1	141.3	346.0	225.4
	3	36.6	73.7	175.6	133.8	353.5	218.3

TABLE 21 Amounts of mycelium in brooms SIAL 169 from Ouro Preto (Brazil), subjected to different regimes of wetting and drying (Text - Table IV.4).

Regime (h wet/dry)	Amount of mycelium (as μg dry wt/mg dried broom)		
	1	2	3
1/23	246.5	200.5	291.5
8/16	264.3	364.8	364.8
16/8	465.3	328.2	456.3
23/1	364.8	328.2	319.2

TABLE 22 Amounts of mycelium in brooms induced at different temperatures on seedlings of Nac x Sca 12, by an isolate of *C. perniciosa* from Pichilingue, Ecuador. (Text - Table IV.5).

Temperature ° C	Amount of mycelium (as µg dry wt/mg dried broom)		
	1	2	3
15	262.0	277.0	262.0
20	354.5	304.2	262.0
25	277.0	304.2	326.8
30	343.7	358.2	365.7

TABLE 23 Sequence of glucosamine assay

Procedure: (i) in vitro

Mycelium dry weight - Three samples of 5 ml mycelial suspension in distilled water

↓

dried to constant weight at 100°C

Glucosamine determination- Three samples of 2 ml mycelial suspension

↓

centrifugation (1500g)

↓

supernatant removed

↓

resuspended in 3 ml 6 N H Cl

↓

hydrolysis in boiling water bath for 6 h

↓

filtration

↓

evaporation

↓

resuspended in 4 ml distilled water

↓

aliquot of 1.5 ml for analysis (reagents)

↓

Absorbance (650 mμ)

(ii) in vivo

Three samples of 25 mg broom tissue

↓

hydrolysis in 3 ml 6 N H Cl in boiling water bath for 6 h

↓

filtration

↓

ion exchange column

↓

TABLE 23 cont.....

(ii) in vivo

↓

evaporation

↓

resuspended in 4 ml distilled water

↓

aliquot of 1.5 ml for analysis (reagents)

↓

Absorbance (650 mμ)

↓

Conversion into fungal biomass.