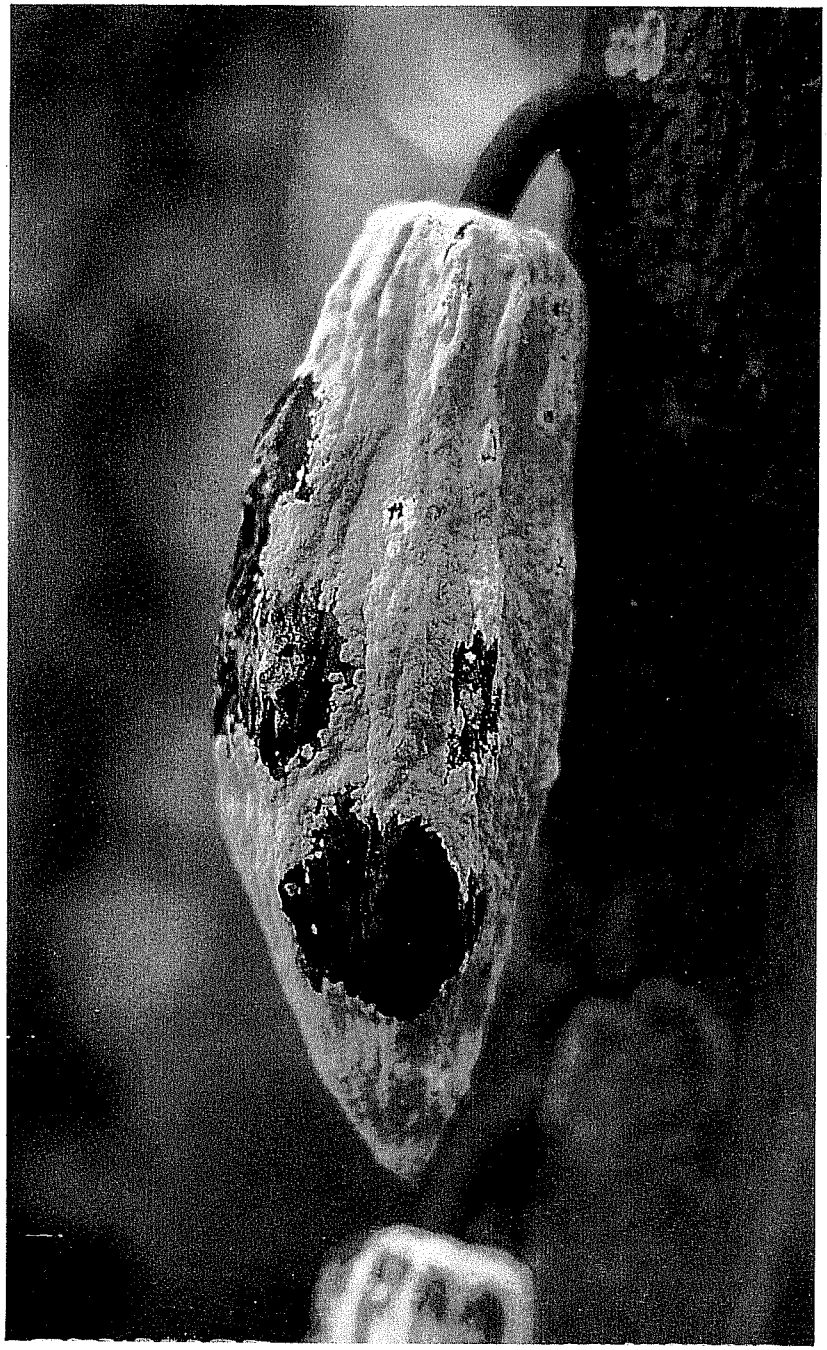

Moniliophthora roreri or frosty pod rot of cacao

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POD ROT OF CACAO CAUSED BY MONILIOPHTHORA (MONILIA) RORERI

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Preface

The statement by Naundorf (1954) — 'all previous work has been made with an incomplete knowledge of the pathogenicity and physiology of the fungus *(Monilia roreri)*, without knowledge of the epidemiology and the conditions favourable for infection' (translation) — is still valid today (Holliday, 1970; Thorold, 1975). Hence the need to assemble all past and present information on this important but basically neglected pathogen in monographic form and to analyze critically this data to obtain a more complete or comprehensive picture of its biology.

I have attempted here to include all relevant published and unpublished data on the disease and the causal fungus. It is not great in contrast to previous papers in this series but it is particularly important to bring unpublished research, usually in the form of inaccessible theses or reports, to light. My three years as cacao pathologist at Pichilingue Research Station have given me access to some of this literature. In my relatively short acquaintance with the fungus, I have investigated its basic life cycle and epidemiology. All the results of this original work are presented here. The fact that one is dealing with a long-term tree crop means that the testing of theories on pathogen-host interactions must span a number of years. Hence a large proportion of the results from recent investigations have not been put to the test. The ultimate test of such research is its use in an efficient, economic control scheme.

There are still gaps in our knowledge of *M. roreri* but I hope that this monograph will serve to highlight the need to pursue further studies of the fungus, not only in the countries affected by the disease, but also in non-cacao-growing countries where greater expertise may provide valuable information on its physiology and biochemistry.

Acknowledgements

Figures 1 and 2 were reproduced by permission of the National Research Council of Canada from the *Canadian Journal of Botany* 56: 2528-2532 (1978).

I wish to thank Mr. P. Holliday for reading and correcting the text and for his initial suggestion concerning its preparation.

The UK Overseas Development Administration (ODA) generously provided funds for this monograph. I am grateful to ODA and to the Instituto de Investigaciones Agropecuarias (INIAP) of Ecuador for providing the opportunity to study this fascinating fungus, and to Mr. D. F. Edwards for his advice and interest.

Finally, I would like to thank Dr. R. A. Samson and other members of the Centraalbureau voor Schimmelcultures for invaluable help with the taxonomy.

Introduction

Despite the increasing and dominant role that petroleum plays in the economies of both Ecuador and Colombia, cacao is still an important export earner or import saver. Both countries now have the ability to process locally most, if not all, of the home cacao production. Colombia is also an importer of cacao, the per capita consumption of this product being one of the highest in the world. The chocolate manufacturers of Colombia have attempted to stimulate interest in the crop over a number of years and have promoted and aided research efforts. However, the interest shown by the manufacturers in Ecuador has only been evident in recent years, following local expansion and the greatly increased capacity to handle the raw product. Increased production can be achieved only by increasing the yields in existing cacao farms and/or by establishing new plantings. The former method is the most efficient in terms of land use, although it is possible that large areas still under forest in the Amazonian region will be used in the future for cacao cultivation.

Pod rot disease of cacao caused by *Moniliophthora roreri* (Ciferri) Evans *et al.* is one of the principal yield-limiting factors in Colombia and Ecuador. It is a potential threat to new cacao-growing areas in these countries as well as to the established industries in those countries in which the disease does not occur. Nevertheless, although the disease was first studied nearly 60 years ago, a further 40 years elapsed before investigations were conducted in Ecuador, at Pichilingue, and in Colombia, at Palmira. The disease has not been studied outside these two countries because, as Baker & Holliday (1957) suggest for witches' broom disease, *Crinipellis perniciosa* (Stahel) Singer, no other important cacao industry has been threatened. Only recently have attempts been made to understand the fungus *per se* and the lack of basic knowledge has undoubtedly hampered efficient control of the disease.

From the external pod symptoms the disease has received several local descriptive names: 'helada' (frost); 'hielo' (ice); 'ceniza' (ashes); 'mal paludico' (malaria); 'pasmo' (wilt due to frost); 'polvilho' (fine dust); 'pringue' (stain); 'pudricion' or 'podredumbre acuosa' (watery rot). It is known also as 'Quevedo' disease, after the town in Ecuador where the disease was first reported (Desrosiers & Suárez, 1974). It is probably most widely referred to in the literature as watery pod rot, but the retention of this name is not recommended as in many instances the presence or absence of internal liquid depends on the cacao type and the age of the pod at infection. As a symptom it is misleading; pods inoculated at a young stage with *C. perniciosa* show similar liquefaction of the bean mass (Evans *et al.*, 1977).

The selection of a common name to describe this disease poses problems. It may be appropriate to use one of the above-mentioned Spanish names but this would tend to be little used or known outside Latin America. Furthermore, on the basis of conidial ontogeny, it is now considered (see Taxonomy section) that the fungus cannot be accommodated in the form genus *Monilia* and the new genus *Moniliophthora* has been erected (Evans *et al.*, 1978).

Hence the commonly quoted names, *Monilia* pod rot and Moniliasis, are technically incorrect. There are obvious dangers in introducing new terminology in the case of a long-established disease and the general reluctance of plant pathologists to accept taxonomic changes has been emphasized by Singer (1976) for witches' broom disease of cacao. However, since the peasant farmers in Colombia and Ecuador identify the pathogen by its frosted or powdery appearance on the pod, the name frosty pod rot has been proposed (Evans *et al.*, 1978): this will be adopted throughout the present text.

I GEOGRAPHIC RANGE

Origins of the disease

During most of the 19th century Ecuador was the leading cacao producer (Wood, 1959) and the Arriba cacao, from the coastal alluvial plain above Guayaquil, achieved fame for its high quality and distinctive flavour. The industry was founded on a single cacao cultivar, Nacional, which is considered to be indigenous to western Ecuador following the discovery of Nacional trees in the understorey of virgin forest (Preuss, 1901; Rorer, 1926). This does not exclude the possibility of deliberate introduction by the Incas. Cacao plantations were seen by the Spanish Conquistadores on the western coast of Ecuador in the early 16th century (Erneholm, 1948; Thorold, 1975). However, the distinctive features and the genetic uniformity of this cultivar suggest a long period of separation from the suspected centre of origin and diversification, thought to be in the upper Amazon basin near to the eastern borders of Colombia and Ecuador (Cheesman, 1944; Purseglove, 1974). Following the introduction of Forastero cacao selections from *c.* 1890 onwards, vast areas of cacao were established under existing forest and by 1914 production had doubled. Van Hall (1914) visited Ecuador during the cacao boom years and described several pod diseases which had been adversely affecting yields over many years. Two types of pod symptoms were distinghished by local names: 'mancha' (lesion) with decay of the whole pod — 'probably caused by a fungus' — and 'helada' producing 'abnormal growth of the pods and beans'. High losses due to the former disease were reported in south-west Ecuador in 1909 and it is possible that the pathogen was *C. perniciosa* which was first observed in Ecuador in this same zone in 1922 (Rorer, 1926). The term 'helada' is still used today by farmers in Ecuador and Colombia as a local name for the second pod rot and is an apt description of the external pod symptoms. Similarly, the first symptom of the disease on young pods is usually a gross swelling or 'abnormal growth'. It would seem, therefore, that Van Hall was unwittingly describing pod rot caused by *M. roreri*.

There is an earlier unpublished report quoted by Jorgensen (1970) that a similar disease — 'most of the pods becoming white while maturing on the tree' — was rampant in a young cacao plantation in the Los Rios province of Ecuador in 1895. However, the first authenticated report of the pathogen and the disease symptoms was not made until 1917 when J. B. Rorer, a plant pathologist working in Trinidad, was sent to Ecuador specifically at the request of plantation owners who were experiencing severe reductions in yield due to an unknown pod malady. Diseased pod samples were sent to R. E. Smith at the University of California and the fungus was identified as a *Monilia* sp., close to *M. fructicola* (Wint.) Honey (Rorer, 1918). Between 1917 and 1925, Rorer made several visits to Ecuador to assess the extent of the disease and to conduct fungicide experiments. In most publications, therefore, Ecuador (notably the Quevedo region) is popularly regarded as the centre of origin of the pathogen. Nevertheless it has been suggested that the disease may have been observed on cacao in Colombia as long ago as 1851 (Holliday, 1953; Baker *et al.*, 1954; Thorold, 1975). The description of the disease is characteristic of *M. roreri* pod symptoms — 'a virulent velvety fungus growth developing to an impalpable dust'.

Whatever the earliest known records of the disease may be, it can be concluded that the fungus is indigenous to the north western region of South America, possibly endemic on *Theobroma* and/or *Herrania* species in Ecuador and Colombia (Rorer, 1926; Holliday, 1970). The introduction of susceptible continuous-cropping Forastero cacao selections to these countries may have brought the pathogen into prominence, as both the Nacional of Ecuador and the Criollo (native) of Colombia have been noted as possessing some resistance to the disease (Rorer, 1926; Wood, 1959).

Present distribution

See CMI Distribution Maps of Plant Diseases No. 13, ed. 3 (1974).

The disease is present throughout the western coastal region of Ecuador, from sea level to *c.* 300m (Aragundi, 1974), but has not been found on the eastern side of the Andean cordillera (Chalmers, 1972a, b, c; Author). The Andes have been an effective barrier to airborne dispersal although, with increasing exploitation and colonization of this still sparsely populated area, the risk of chance introduction through infected pod material is high. Similarly in Colombia the disease is restricted to the western and north western cacao-growing zones of the country, from sea level to over 1000m (Baker *et al.*, 1954; Wood, 1959). In both countries the range of climatic conditions under which the disease occurs is wide, particularly rainfall which varies from 40cm (with irrigation) to 1000cm per annum. In Venezuela, the disease is restricted to the region south of Lake Maracaibo — in the western Andes and in the river Catatumbo area of Zulia state — in the extreme west of the country (Palma, 1949; Malaguti & Diaz, 1958; Wood, 1959; Reyes, 1975). In Peru the disease has been reported from several isolated valleys in the Andean cordillera: Jaen (Cajamarca department), Tingo Maria (Huanuco department) and Convencion (Cuzco department) (McLaughlin, 1950). The disease was not identified, however, in the former two areas during a later survey (Burgos, 1954a, b). Orellana (1956) reported the disease in eastern Panama in the Darien province close to the Colombian border, and more recently *M. roreri* has been identified on cacao in the Cahuita zone of Costa Rica, close to the Panamanian border (Enríquez & Suárez, 1978). All evidence indicates that it has been present for a number of years. There is also an unsubstantiated and apparently erroneous report that the disease is present in northern Brazil (Diaz, 1957) (see also Wellman, 1972). A suspected case of frosty pod rot was investigated recently in the very important cacao-growing Brazilian state of Bahia but the fungus proved to be a hyperparasite *(Amblyosporium* sp.) of *Botryodiplodia theobromae* Pat. colonizing old unharvested pods (Author, unpublished). In the Alto Beni region of Bolivia, pod disease has been attributed mainly to frosty pod rot (Anon., unpublished); however, during the author's recent visit *M. roreri* was not encountered and a close examination of infected pods revealed that the disease was caused by *C. perniciosa; M. roreri* having been diagnosed on the basis of the internal watery rot.

It is of interest to note that the disease is still confined to the north western part of South America and to the southernmost tip of Central America. Whether or not this distribution is the result of completely natural spread or aided by man, as considered by Orellana (1956), is difficult to determine. It is perhaps significant that the only part of Venezuela affected so far is separated from the coastal region of Colombia by the narrowest part of the Andean cordillera. It is possible, therefore, that the present distribution of the disease is the result of unchecked airborne spore movement along the western side of the Andes. In Colombia, Ecuador and Peru it is present in all the cacao areas outside the Amazon basin. According to Chapman (1926), the elevation of the Andes has not only separated tropical eastern from tropical western Ecuador but has divided the humid tropical zone of South America into apparently disconnected cis— and trans-Andean areas. Thus the Andean uplift has closed the dispersal routes between Amazonia and the Pacific coastal region whilst contact has been maintained between the latter and Central America. Geographically, therefore, the pathogen may be reaching the limits of its natural distribution. Further dispersal by man is possible and the disease is a potential threat to other cacao-growing countries (Holliday, 1971; Thurston, 1973). The long period from infection of a pod to lesion formation and sporulation emphasizes the risk of transporting planting material via the pods and suggests how man could be important as a carrier of *M. roreri*. It is particularly ironic that eastern Ecuador, the previous scene of cacao collecting

expeditions, is receiving improved cacao hybrids from western Ecuador. If the pathogen is introduced it may prove to be disastrous for future hopes of exploiting Amazonia for cacao cultivation (Wray, 1954).

II HOST RANGE

During his survey of frosty pod rot of cacao, Rorer (1918) also recorded the disease on *Theobroma bicolor* Humb. & Bonpl. and *Herrania balaënsis* Preuss, two species commonly planted around dwellings in western Ecuador. The latter species also occurs in the forests and is a possible wild host of *M. roreri*. The fungus has also been found on wild *T. gileri* Cuatr. in north-west Colombia (Baker *et al.*, 1954) which strengthens the theory that the pathogen is endemic to that region (Holliday, 1970). Despite these earlier reports some later reviews have stated that other hosts of *M. roreri* are unknown (Ampuero, 1967).

A collection of *Theobroma* and *Herrania* species is maintained at the Pichilingue Research Station and has enabled a detailed study to be carried out of the host range. Pods of the following species have been shown to be susceptible to the pathogen as a result of natural infection and/or inoculation: *T. angustifolium* Moçino & Sessé, *T. bicolor*, *T. mammosum* Cuatr. & León, *T. simiarum* Donn. Smith, *T. sylvestre* Mart., *Herrania balaënsis*, *H. nitida* (Poepp.) Schultes, *H. pulcherrima* Goudot. and 4-5 unidentified *Herrania* spp. All species in the collection have been observed with infected pods and it seems likely, therefore, that the potential host range extends to all species of the genera *Theobroma* and *Herrania*.

III TAXONOMY AND MORPHOLOGY

The fungus was named *Monilia roreri* Ciferri (apud Ciferri & Parodi, 1933) in honour of J. B. Rorer who had pioneered research investigations. Specimens from Ecuador and Colombia were examined and the species was thought to be close to *M. seaveri* Reade. The absence of a sexual stage was noted but it was considered to represent the imperfect state of a *Sclerotinia* (Ascomycotina). Wellman (1972) classified frosty pod rot amongst the sclerotial diseases and described the mummified pod as sclerotioid. No further work on the taxonomy of *M. roreri* has been carried out.

A brief description of the fungus given by Holliday (1970) was a slightly modified form of the original by Ciferri. However, *M. roreri* has since been redescribed and the results of this latest taxonomic analysis, with *in vitro* and *in vivo* observations (Evans *et al.*, 1978), are presented in full.

The fungus *in vitro*

The fungus can be isolated from infected pods by surface sterilization (2.5% sodium hypochlorite for 1 min), followed by washing in sterile water and transfer to potato dextrose agar (PDA) (López, 1954). But by simply removing the outer pod epidermis and plating the underlying tissues directly on agar excellent results are obtained.

Growth on malt extract agar slow, colony attaining a diameter of 8-15mm after 2 weeks. Margin appressed to slightly raised, even. Mat typically woolly to felty, at first Pale Ochraceous Salmon or Pinkish Buff, finally becoming Cinnamon Buff to Clay Colour or locally Wood Brown to Apricot Buff (Ridgway, 1912). Occasionally the mat is initially downy to farinaceous, white to cream or with ochraceous tinges. Odour indistinct, reverse unchanged. Laccase and tyrosinase have been identified: 0·1 M α-naphthol in 96% ethanol turns purple when laccase is present; 0·1 M p-cresol in 96% ethanol changes to orange-brown with tyrosinase.

Advancing zone: hyphae hyaline, thin-walled, septate, sometimes slightly irregular with swellings, 1·5-5 μm wide. Aerial hypha of two types: (a) as in advancing zone but with slightly thickened walls, and (b) hyaline to pale brownish, thick-walled, non septate, (1-) 1.5-2 (-3) μm wide, skeletoid, rarely

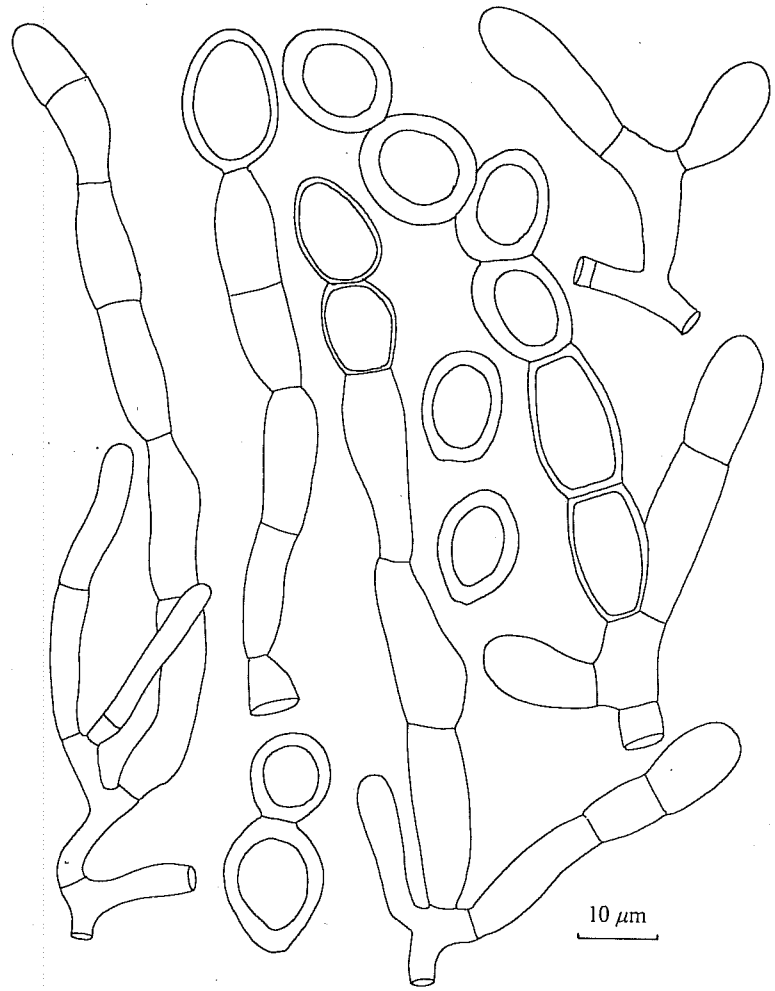


Fig. 1. Conidial structures of *Moniliophthora roreri*.

branched. Conidiophore branched, giving rise to a basipetally maturing chain of conidia. Conidia easily separable, thick-walled, pale yellow, brown en masse, typically globose to subglobose (6.5-) 8-15 (-25) μm diam., sometimes ellipsoid, 8-20 × 5-14 μm, wall up to 2 μm thick. Cylindrical thin-walled conidia also occur, probably derived from immature chains. Submerged hyphae as in the advancing zone, but swellings more frequent. One strain formed chlamydospores or chlamydospore-like swellings. Crystals present, tetragonal. No sexual stage has been reported.

M. roreri also grows well on sterilized barley grains (Suárez, 1971) and liana cuttings. The significant loss in weight of these substrates suggests the presence of cellulases and ligninases. Compact, cream-coloured clumps of sterile mycelium with yellow exudate droplets are frequently formed and red-purple patches, composed of heavily incrusted red hyphae, occur in old cultures. Growth temperatures are optimum 25-26°C, maximum 33°C.

The fungus *in vivo*

According to Suárez (1971, 1972), the fungus invades green pod tissues intercellularly with the internal production of conidiophores and conidia. Examination of sections of cacao seedling stems shows that *M. roreri* grows between the parenchyma cells of the cortex in the form of characteristic swollen, 4.7 μm diam., lumpy or convoluted hyphae. In dead or dying host tissues, the mycelium is similar to that produced in culture.

Conidiogenesis and septal structure

Attempts to observe conidiogenesis by means of time-lapse studies have not been successful due to the slow growth of the fungus in culture. However, from light and scanning electron microscope observations, it appears that the conidiogenous cell is first differentiated from a vegetative hypha by the swelling of the apex and the development of the primary holoblastic conidium, after differentiation of which a segment of the fertile hypha below its basal septum often swells and differentiates into the next propagule. All conidia are formed in the same manner thus resulting in a basipetally-maturing chain of conidia (Fig. 1; Fig. 2a-d). With time, the conidia tend to round-off and become thick-walled and light brown in colour. In transmitted light, the wall is seen as an opaque refractive area suggesting the presence of chitin. The chain usually consists of 4-10 conidia, enclosed or enveloped within the original cell wall. The conidia are subsequently liberated by fracture of this wall. After conidial secession, remnants of the wall may adhere to the conidia (Fig. 2e).

The vegetative mycelium contains septa with typical dolipores (Fig. 2f) as previously reported from basidiomycete hyphae (Moore & McAlear, 1962). Hence the fungus represents the asexual stage of an unknown basidiomycete.

The type species of *Monilia, M. fructigena* (Pers. ex Fr.) Westend. is the anamorph of the discomycete *Monilinia fructigena* (Aderh. & Ruhl.) Honey. The conidia of *Monilia* species are formed holoblastically and mature acropetally. Hence, *M. roreri* cannot be considered to be congeneric with *M. fructigena*. Since it is distinct from any of the basidiomycete asexual stages so far described, the new genus *Moniliophthora* Evans *et al.* (1978) has been proposed, *M. roreri* being designated the type species, with the following generic description: hyphae hyaline, septate, without clamp connexions but with dolipores. Conidia in chains, globose to ellipsoidal, formed in basipetal succession.

Growth requirements

Little is known of the basic nutritional requirements of *M. roreri* and much remains to be done in this direction.

Barros (1975a) studied the effect of pH on growth in both solid and liquid media. Growth of the mycelium occurred from pH 3.5 to 8.0, optimum 5.5, whilst maximum spore production was at pH 7.0. All the media used increased

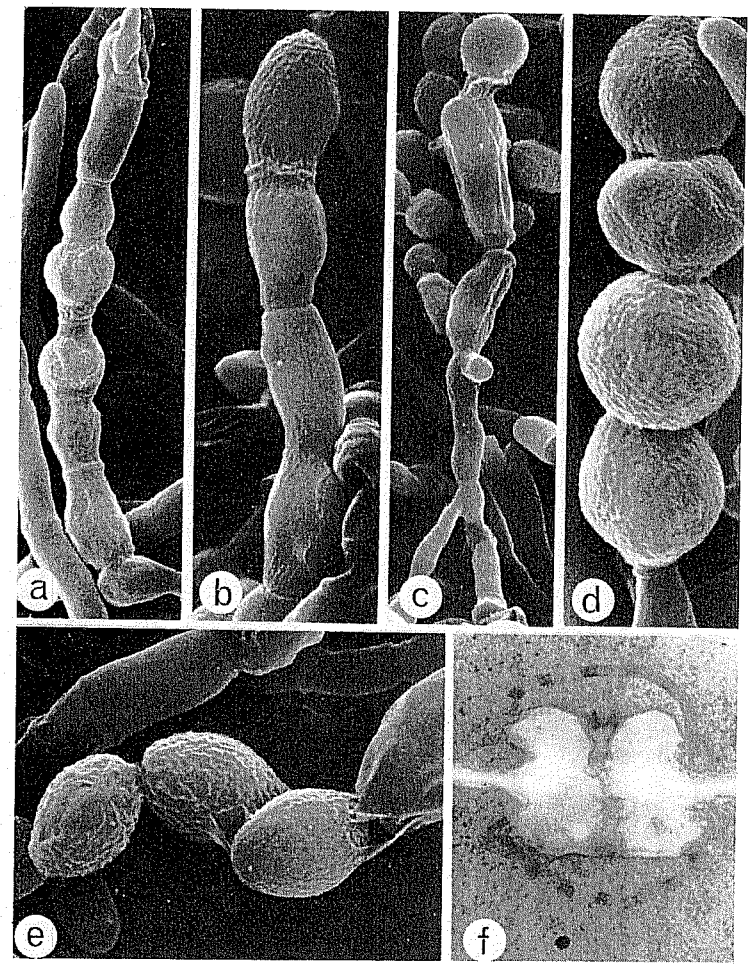


Fig. 2. a-e, SEM micrographs of various conidial structures showing basipetal chains of conidia (a × 1900, b × 2700, c × 1400, d × 4100, e × 2600); f TEM micrograph showing dolipore septum in the vegetative hyphae (× 60,000).

in alkalinity with time. At the lower pH levels the mycelium was abundant with atypical, white, floccose colonies.

Physiological studies of the fungus in living tissues and in dead tissues or in culture are necessary to elucidate the mechanism of pathogenicity and it is thought that two separate nutritional states occur: the biotrophic or parasitic state and the necrotrophic state. In terms of nutrition, *M. roreri* should be regarded as a hemibiotroph (Cooke, 1977).

IV DISEASE LOSSES

Economic considerations

The much quoted example of the effect on cacao yield of frosty pod rot in Ecuador is still worth repeating here (Table 1). From the first appearance of the

disease in epidemic proportions (*c.* 1916) until the arrival of witches' broom disease (1922-1925), annual losses in Ecuador have been estimated at 9-10 000 tons of dry cacao (Jorgensen, 1970) or 20-30% of the total production. Nearer to the western slopes of the Andes, Rorer (1918) put pod losses due to *M. roreri* as high as 95% of the crop. The impact of frosty pod rot on the cacao industry of Ecuador was summed up by Rorer (1926): 'The sudden drop in exports in 1918 and the succeeding years until 1923 was due to the ravages of the *Monilia* disease in the plantations of Venezuela cacao, many of which were completely

Table 1. Annual yield of a plantation of 35 000 Trinitario cacao trees in Ecuador (After Rorer, 1926)

Year	Quintales*	Observations
1916	650	'Monilia' not present
1917	710	'Monilia' not present
1918	226	'Monilia' appeared
1919	36	'Monilia' continued
1920	—	Plantation abandoned and replaced by bananas.

*1 Quintal = 101·4 lbs.

abandoned'. The combined effect of the two diseases caused a decline in the cacao industry and diversification into other crops, particularly bananas. The vast cacao haciendas and plantations have all but disappeared and the cacao production is now mainly in the hands of the smallholder who has few overheads.

More recently a disease loss survey has been made of all the representative cacao-growing zones in western Ecuador (Aragundi, 1974) and preliminary results are shown in Table 2. Confusion of pod symptoms between *M. roreri* and *C. perniciosa* may not accurately reflect the actual losses due to frosty pod rot but the figures indicate the overall high yield reductions as a direct result of pod diseases in Ecuador. The peasant farmer there, whose cacao is frequently his only source of ready income, has had to learn to live with severe reductions in yield due to disease. Annual variations in disease losses can thus directly affect his standard of living.

Table 2. Disease losses in the principal cacao zones of western Ecuador, 1972-73 (Modified from Aragundi, 1974)

Zone	Yield	Percentage pods diseased		
		Moniliophthora	Total diseased	Rainfall (mm)
Viche	676*	40·5	51·2	2135
Chone	420	40·4	67·8	1175
Quevedo	1292	21·6	35·9	2057
Montalvo	467	43·6	65·6	—
Milagro	1592	20·3	26·5	1076
Machala	2281	30·1	32·3	389

* lbs dry cacao per hectare.

The effect of frosty pod rot on yield in Colombia is probably easier to assess since in that country *M. roreri* and *C. perniciosa* have tended to be complementary in their distribution and rarely overlap (Baker *et al.*, 1954). Barros (1961, 1966) estimated a 30-40% decrease in the production of dry cacao due to the disease,

with losses in 1973 calculated at 7000 tons or the equivalent of 6 million dollars (Barros, 1975a).

Frosty pod rot has been described as serious in Peru (McLaughlin, 1950; Desrosiers & Diaz, 1957) but, as in Panama, cultivation of the crop is not extensive. Similarly in Venezuela the disease is confined to the western region where less than 2% of the country's cacao is grown and therefore, at present, it is not economically important.

Climatic considerations

According to a survey carried out in Ecuador by Fowler *et al.* (1956), frosty pod rot is not significantly negatively correlated with yield (Table 3). One explanation of this lack of relationship was explained by an insufficient variation in the amount of the disease between farms. For example, in Table 2 it can be seen that in the Machala area the incidence of *M. roreri* is high but the actual yield is far in advance of any of the other zones. Pressures on the potential yield may be less, due to local conditions. The extremely low rainfall in this area is supplemented by irrigation. It should be noted that yield is difficult to interpret when dealing with cacao diseases because of the difference between potential yield, the theoretical yield resulting from the number of pods set, and actual yields, from those healthy pods remaining at harvest. Cacao is subject to physiological wilting of young pods (cherelles) in addition to pod diseases. In most instances the losses due to disease are expressed as a percentage of the total pods harvested and ignore those lost in the cherelle or immature stage either through disease or natural processes within the tree.

Table 3. Correlation coefficients showing the associations between *Moniliophthora* pod rot disease and four variables observed in 146 cacao plantations in western Ecuador (After Fowler *et al.*, 1956)

Variable	*Moniliophthora* pod rot disease
Yield	−0·09
Rainfall	+0·38**
Sunlight	+0·35**
Witches' broom disease	+0·34**

**Significant at the 1% level.

In general, high disease losses and low yields are associated with high rainfall areas. Fowler *et al.* (1956) showed a significant positive correlation between frosty pod rot and witches' broom which they interpreted simply to mean that both diseases are favoured by high rainfall (Table 3). Another significant positive correlation was demonstrated between the disease and the amount of sunlight. This apparently anomalous result may be because the most uniformly shaded cacao plantations (less sunlight) occur in the low rainfall areas whilst the unevenly shaded plantations (high sunlight) are established under existing forest trees in the high rainfall areas. Diaz (1957) also indicated that rainfall and exposure to sunlight had some effect on the incidence of pod rot in shaded and unshaded trees at Pichilingue (Table 4). In the year of low rainfall disease losses were lower in the shaded cacao and it was proposed that dew formation on exposed trees may have been contributory by providing conditions favourable to pod infection. But the results are so variable and incomplete that it is impossible to reach a definite conclusion.

The effect of rainfall on pod losses due to *M. roreri* in selected cacao cultivars in a wet and a dry zone of western Ecuador is summarized in Table 5. The wet Santo Domingo zone (altitude 300m) is near the foothills of the Andes and extremely high disease losses have largely led to the abandonment of cacao

Table 4. Disease losses due to *Moniliophthora* pod rot in shaded and unshaded cacao at Pichilingue (After Diaz, 1957)

Year	Percentage Pods Diseased		Rainfall (mm)
	Shade	No shade	
1951	36·9	37·7	2725
1952	28·5	30·9	1480
1953	34·7	33·8	3040
1954	17·9	27·4	1603
1955	29·3	29·7	2123

cultivation. It should be re-emphasized that the figures given in Table 5 are almost certainly over-estimated for pod losses due to *M. roreri*.

Table 5. Comparison of losses due to *Moniliophthora* pod rot disease in various cacao cultivars in a high rainfall (SD) and a low rainfall (V) zone in western Ecuador (Modified from Anon., 1974).

Cacao cultivar	1969		1970		1971		1972		1973	
	SD	V	SD	V	SD	V	SD	V	SD	V
EET-48	52*	4	76	4	65	8	35	24	36	8
EET-62	54	17	79	10	70	3	20	14	41	6
EET-103	67	3	81	4	57	7	36	26	34	6
EET-400	62	0	73	10	60	4	34	14	33	9

*Pod losses due to *M. roreri*. Percentage of total crop.
SD — Santo Domingo, mean annual rainfall 4200 mm.
V — Vinces, mean annual rainfall 1500 mm.

Monthly percentages of pods infected with *M. roreri* have been positively correlated with the amount of rain in the fourth preceding month, both in Ecuador (Desrosiers *et al.*, 1955) and Colombia (D. Brown, personal communication). This was considered to be the result of pod infection at an early stage.

V THE DISEASED PLANT

Until recently, the pods were the only tissues reported as being attacked by the fungus (Ampuero, 1967; Desrosiers & Suárez, 1974; Thorold, 1975). However, inoculation experiments at Pichilingue have shown that the pathogen can infect and produce characteristic symptoms in seedlings and unhardened stems or flushes.

Pod infection

1. Inoculation studies

Rorer (1918) mentioned that planters in Ecuador had tried unsuccessfully to infect healthy pods with conidia of *M. roreri*. Likewise, his own inoculations of pods in the field gave negative results, although under laboratory conditions several inoculations were successful. Almost certainly these pods had been naturally infected beforehand, as laboratory pod inoculations cannot be done due to the long incubation, some 6-10 weeks. This may also account for the failure of the field inoculations, as his visits to Ecuador were short, 'It seems that it would be necessary to spend 3-4 months in experimenting in order to obtain good results from inoculations... but unfortunately the author has not this time at his disposal' (Rorer, 1918), and for the conclusion reached by Bastidas (1953) that *M. roreri* is not the real pathogen but merely a saprophyte on diseased cacao pods. The latter worker used eleven different inoculation

methods on more than 3000 pods of various ages and never obtained a single infected pod. All experiments were conducted in the laboratory, hence the lack of success. It is worthwhile noting that from Rorer's early work until the 1950s Koch's postulates had not been satisfied.

Naundorf (1954) in Colombia inoculated attached pods with a spore dust of *M. roreri* but the difference in the amount of infection between treated and control pods was small and led him to conclude that the mere presence of inoculum is not sufficient for infection and that penetration is aided by wounds (see Table 6). One of the commonest casual agents of pod wounding in the Cauca valley of Colombia is the Pentatomid stink bug, *Mecistorhinus tripterus* F., and this was enclosed in cheesecloth bags placed around cacao pods dusted with *M. roreri* conidia. A high proportion of these pods later developed infections in comparison to pods inoculated with spores alone (Table 6). These results were corroborated by Sepulveda (1955) and later by Franco (1958). However, the latter worker also concluded that significant infection can occur without the intervention of insects. Diaz (1957) in Ecuador wound-inoculated pods at the base of the peduncle or laterally on the pod wall and obtained infection rates superior to the control. With older pods there was some evidence that pod infectivity decreased with pod age. Bejarano (1961) employed a simple but effective inoculation technique and was the first worker to demonstrate that prior pod wounding was not necessary for high infection. Spore suspensions were sprayed on unwounded and wounded pods enclosed in plastic bags. Pods of all ages were susceptible to infection but older pods (> 14cm) had appreciably less infection. Using the same method Sotomayor (1965) obtained consistently high infection rates with a very concentrated spore suspension. An even simpler method, whereby a cotton-wool pad is dipped into a spore suspension and placed directly on the pod surface, was successfully used by the author to test spore viability. In Table 6, a comparison is made of all the inoculation methods employed and the rates of infection obtained. In most experiments, where free water was not available, infection was low and merely by retaining a damp pod surface, as with the cotton-wool pad method, consistently high infection was achieved.

Table 6. Inoculation methods employed to test the pathogenicity of conidia of *M. roreri*. (Modified from various authors)

Author	Inoculation method	Number of of pods inoculated	Percentage infection*	Pod age or size at inoculation
Rorer (1918)	Dry conidia	46	0	range
Bastidas (1953)	Various laboratory methods	3372	0	range
Naundorf (1954)	Conidial dust	875	8	range
	Conidial dust	100	17	range
	Dust + Pentatomids	100	62	range
Franco (1958)	Conidial dust	85	16	30-45 days
	Dust + Pentatomids	91	60	30-45 days
Diaz (1957)	Wound-inoculated peduncle	42	48	1-6 cm
	Wound-inoculated pod	12	53	1-6 cm
Bejarano (1961)	Sprayed spore suspension			
	Wounded pod	60	46	4-8 cm
	Unwounded pod	60	38	4-8 cm
	Unwounded pod	180	47	All ages
Sotomayor (1965)	Sprayed spore suspension (v. high concentration)	2200	91-100	80 days
Evans	Cotton-wool pad + spore suspension	700	92	30-40 days

*After deduction of control infections, wilts not included. All using "fresh" conidia, less than 1 month old.

2. Inoculum density

Sotomayor (1965) used an extremely high spore concentration, 35 million spores/ml water, to obtain maximum infection. More realistic field inoculum densities can be tested with the cotton-wool pad technique. From preliminary experiments it appears that at very low concentrations (10 conidia/pod) infection is slight both in terms of quantity and symptoms produced. At 100 spores/pod almost 60% infection resulted and at greater concentrations (>100) almost all the pods became infected with accompanying gross swellings and distortion (D. K. Cronshaw, personal communication). More work is needed in this direction to establish if symptom expression and successful fungal invasion are related to the inoculum density, particularly if pod inoculation is to be used as a tool to test resistance to the pathogen (see Fig. 3).

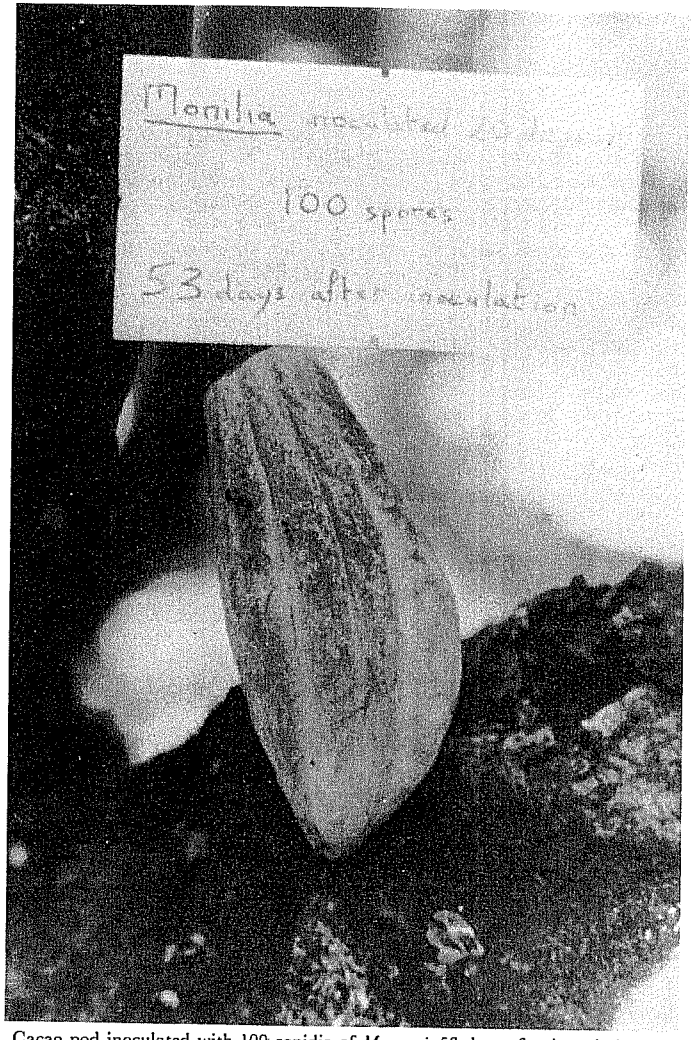


Fig. 3. Cacao pod inoculated with 100 conidia of *M. roreri*, 53 days after inoculation. Note gross lateral swelling.

3. Germination and penetration

The actual mechanism of pod penetration was not investigated until Suárez (1971, 1972) demonstrated that the conidia of *M. roreri* germinate and penetrate the pod at all stages of development, directly through the epidermis or via stomata without the presence of wounds. On the pod surface spore germination occurs within 3 hours by 1-5 germ tubes which extend over the epidermis and produce fine infection hyphae. These appear to penetrate the epidermis near the base of the glandular hairs. Eight hours after inoculation short, thick hyphae and thinner non-septate hyphae with homogeneous contents can be seen growing intercellularly beneath the epidermis. Branching conidiophores and conidia develop later within the cortex. Chlamydospore-like bodies were also reported to be present in the outer pod layers.

The only detailed study of spore germination was made by López (1954) but, as will be seen later, there appears to be no relationship between germination in the laboratory and pod penetration and infection in the field. López found that with 'mature' spores (>3 days old) very low germination rates were obtained and in most cases they did not germinate. 'Young' conidia (1-2 days old) were then employed in the germination tests. Twice-distilled water and 1% soil extract gave the greatest percentage germination (Table 7). Germination occurred at pH 4-8 with the optimum at pH 6 and in the absence of free water, at 80.5-100% relative humidity. Light was not critical and although the direct effect of temperature was not investigated, it was noted that the highest germination rates were obtained during the night (at 22-24°C) compared with those from experiments conducted during the day at high temperatures (up to 32°C).

Table 7. Effect of nutrients on the germination of 1- to 2-day-old conidia of *M. roreri* (Modified from López, 1954)

Medium	Percentage germination			
	Hours			
	4	8	12	22
1% Saccharose	—	16	24	M
2% Saccharose	—	9	12	16
4% Saccharose	—	—	—	—
1% Glucose	2	7	10	13
2% Glucose	1	2	4	5
4% Glucose	—	3	3	4
5% Healthy pod infusion	1	18	27	M
5% Infected pod infusion	—	5	7	9
2% Cacao flower infusion	2	10	19	M
2% Bacto-peptone	—	7	13	25
Distilled water	—	14	22	22
Twice-distilled water	8	45	67	M
1% soil extract	—	32	50	M

— No germination.
M Mycelial growth, germination rate not recorded.

The results of these studies have been interpreted by various workers to mean that the conidia have a short life but, as Bejarano (1961) and the author have observed, germination tests are not necessarily related to pathogenicity since spores which failed to germinate in the laboratory nevertheless proved to be infective following inoculation of pods in the field. There may be a dormancy mechanism in older thick-walled conidia.

During a study of the air-spora of cacao farms at Pichilingue a low percentage (8-10%) of trapped conidia of *M. roreri* were found to germinate directly on the vaseline-coated slide on the night of capture. An analysis of the relevant meteorological data for the days during which this behaviour was recorded

showed that a combination of low temperatures (daily mean 21-22°C) and high humidity (daily mean 91-93%) favour spore germination. Early morning drizzle or 'garua' also occurred over this period which is in the middle of the long dry season. It is possible that the dormancy was broken by the changes in conditions.

4. Symptoms

a) On cacao pods

One of the characteristics of frosty pod rot, which it shares in common with witches' broom, is the long incubation from penetration to the appearance of symptoms. This is modified to some extent by the age of the pod at inoculation and the cultivar (Sotomayor, 1965). For this reason, some of the results obtained by Naundorf (1954) are difficult to interpret as infection was recorded 4 weeks after inoculation, but experience in Ecuador has shown that observations must be maintained for much longer periods and that lesions never appear during the first month (see Tables 10 and 11).

The most thorough study of pod infection was carried out by Suárez (1971) and the results are summarized by Desrosiers & Suárez (1974):

> Pods inoculated at 20, 40 and 60 days. The entire process of penetration, intercellular and intracellular invasion (symptom appearance) takes place in 40 days. This results in a necrosis of the fruit which especially in pods inoculated at 20 and 40 days, may easily be confused with physiological wilt.
>
> Pods inoculated at 80 days and some at 60 days. The infective process, penetration to intracellular invasion, occurs at 60 days. These pods present symptoms of premature ripening (the appearance of areas of mature coloration on pods which are immature), and deformation without externally visible necrosis. Some pods may appear healthy externally but may be totally destroyed within.
>
> Pods inoculated at 120, 140 and 160 days. In these pods infection develops more slowly. Small localised lesions may develop on the surface after 60 days. In many cases, the endocarp is not penetrated and the seed from infected pods may be salvaged.

A single cultivar was tested in this investigation and it is necessary to add here the results obtained by other workers. Naundorf (1954) similarly concluded that there was a relationship between the presence of the disease and the wilting of young pods. He found that the amount of wilt was greatly increased in cherelles inoculated with *M. roreri*. However, Bejarano (1961) utilized pods from 0.5 to 14 cm in length (7-69 days old) in his inoculation studies and noted no differences in the degree of wilting between inoculated and control pods. For spore viability tests the present author inoculated over 4000 cherelles and there was no correlation between wilt and the presence of the pathogen. The most obvious, consistent symptom resulting from cherelle inoculations is the appearance of conspicuous swellings, often causing distortion, lighter in colour than the rest of the pod surface (Fig. 3). In this study 20-100% of the infected pods developed swellings 35-85 days after inoculation (Table 11). The swellings, identical in appearance to those caused by *Crinipellis perniciosa,* remain green and fully turgid long after the cherelle has wilted (Fig. 4); this in itself suggests that the fungus is not the cause of wilt.

Irregular and premature ripening may precede lesion development, which occurs 53-65 days (Sotomayor, 1965), 30-89 days (Bejarano, 1961), 43-90 days (Tables 10 and 11) after inoculation. Chocolate to dark brown irregular lesions gradually coalesce to cover the whole pod surface, although in pods infected at a later stage a single sunken dark brown lesion often predominates. White mycelial growth, in the form of a pseudostroma, and sporulation are usually rapid, some 3-8 days after lesion appearance (Bejarano, 1961; Sotomayor, 1965; Author). The spore bloom or powder is cream-coloured at first but changes to

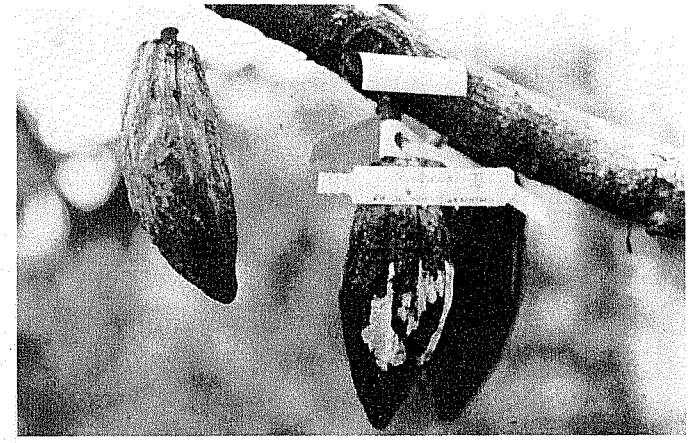
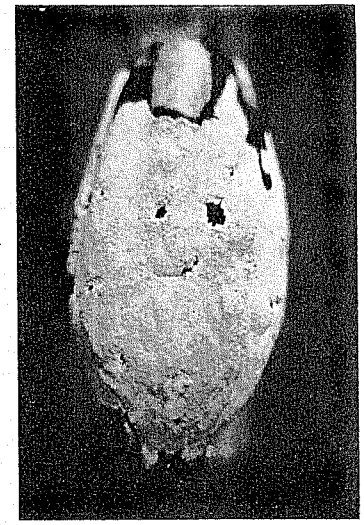


Fig. 4. Cacao pods 60 days after inoculation with 9-month-old conidia. Note white pseudostroma on one pod. The other pod has wilted but the inoculated area has remained green and turgid.

grey, tan or brown. The common names quoted earlier are descriptively accurate of the advanced pod symptoms (Figs. 5 and 6).



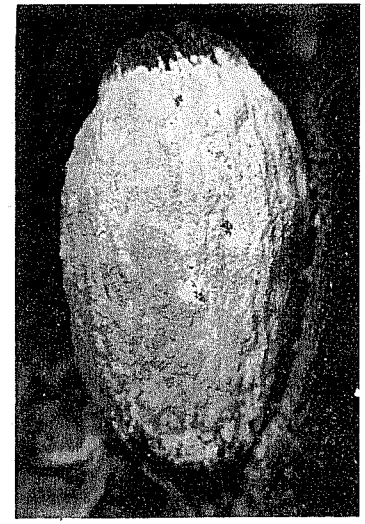


Fig. 5. Naturally infected cacao pod almost completely covered by the pseudostroma which is forming the spore bloom.

Fig. 6. Naturally infected cacao pod several weeks after sporulation. The spore mass has darkened and is flaking. Note spore dust on adjacent bark.

Internal symptoms also vary with pod age. Pods infected at the cherelle stage show failure of bean development, the tissues being replaced by disorganized gelatinous substances or liquid, hence the name watery pod rot. Others reveal

complete destruction of the bean mass and only a moist or even a dry rot. In later-infected pods the beans are stuck together and to the pod wall, making them difficult to remove. Such pods are often noticeably heavier than healthy pods of similar size.

b) On *Theobroma angustifolium*

Trees at Pichilingue have shown heavy pod losses due to frosty pod rot. Cross-inoculations of cherelles, using conidia from diseased cacao pods, have shown that few symptoms are visible until *c.* 3 months after inoculation. Distinct lesions are not evident, and are probably masked by the colour and texture of the pod surface, but the typical cream pseudostroma is present (Fig. 7). In most cases the pods seem healthy but are abnormally light and when

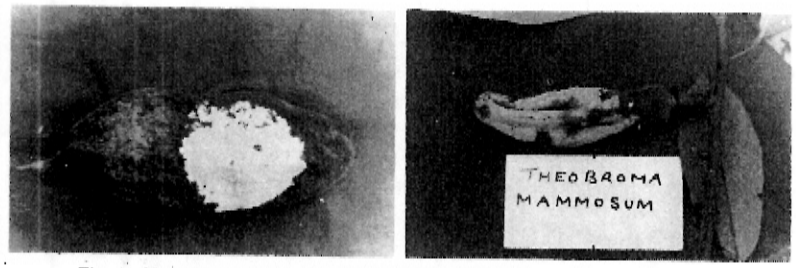


Fig. 7. Naturally infected pod of *Theobroma angustifolium* with pseudostroma.
Fig. 8. Naturally infected pod of *Theobroma mammosum* beginning to mummify.

opened the shrunken bean mass is densely covered with a cream or orange pseudostroma bearing grey-brown spore blooms. Later these pods become very brittle, the walls crack or rupture and the dry conidia are released. *T. mammosum* (Fig. 8), *T. simiarum* and *T. ? sylvestre* show similar pod symptoms to those described above.

c) On *T. bicolor*

Distorted or swollen pods may be observed on the tree but lesions are rare. However, after infected pods fall, chocolate brown lesions develop within 8 days, followed by light sporulation. The woody pod tissues seem to be unfavourable to rapid mycelial growth and dense spore production. Complete destruction of the bean mass is not common and internal necrosis may take the form of a wet or dry rot.

d) On *Herrania*

Symptoms vary according to the species but generally the first evidence of infection is a small, chocolate to dark brown, lateral lesion, followed by a dry necrosis of the whole pod. Extensive external sporulation is uncommon but, as with *T. angustifolium*, the bean chamber and contents are covered with a thick cream-brown mycelial skin producing masses of spores.

Flower infection

Naundorf (1954) inoculated newly pollinated cacao flowers with dry conidia and found that only 16-22% of these flowers later set fruits compared with 62-71% for the controls. Bejarano (1961) expanded this work and inoculated cacao flowers with dry conidia or a suspension either before, after, or at the same time as pollination. Pod set was recorded and the pods were observed to maturity. Results were variable. In one experiment there were no significant differences in the levels of pod setting in any of the treatments, including controls. In others there was significantly lower fecundation in the flowers inoculated before or at pollination compared with the controls. In the three inoculation treatments 11-50% of all the pods which set later became diseased,

although no external symptoms (e.g. swellings) were visible until lesion formation. It was suggested that the conidia either immediately penetrate the ovary or that they remain on the dry flower parts and invade the developing cherelle.

Suárez (1971) repeated these experiments with negative results. Tissue samples from ovaries and cherelles were examined but mycelium of *M. roreri* was not detected. It would seem, therefore, that this important aspect is still unresolved and to avoid further ambiguous results such experiments should be conducted on trees grown under greenhouse conditions, free from contamination.

Seedling infection

1. Inoculation

In an attempt to determine whether or not the fungus could penetrate and colonize non-pod tissues, a simple seedling inoculation test was devised, based on that of Holliday (1955) for *C. perniciosa*. Three- to five-day-old seedlings were immersed in a conidial suspension for 5 min and planted directly in seed boxes, with uninoculated control seedlings forming the borders. The suspension was prepared by washing the surface of a 15- to 30-day-old barley culture of *M. roreri* with sterile distilled water. Care was taken to water each seedling individually to avoid splash or run-off and contamination of the controls. Several cacao cultivars and other *Theobroma* species were tested.

2. Symptoms

a) Cacao

Symptoms are variable within and between cultivars but the most consistent symptom of inoculated seedlings is the rapid or premature formation of bark tissues, contrasting with the smooth green stems of control plants.

Individual seedlings may develop acute signs of infection within 3 weeks, the lower stem or hypocotyl swells, often grossly, and is frequently covered with patches of cork cells (Fig. 9). Cotyledons fail to open and such seedlings invariably wither and die, 4-5 weeks after inoculation. In others the lower stem is stunted and swollen and, although these plants usually survive, they remain significantly smaller than the controls. In all susceptible seedlings the root systems are well developed and there is some evidence of root stimulation in the early stages of growth. However, in the majority of seedlings no symptoms are visible until 6-8 weeks after inoculation, when a complex range of stem disorders becomes evident, including premature and irregular bark formation, basal stem thickening or distortion, stunting, rippling, undulations or discrete swellings, abnormal lenticels or masses of cork cells. The proportion of seedlings with symptoms increases with time and by the end of the fourth month most of the inoculated plants can be readily distinguished from the controls on the basis of lower stem abnormalities. The hardening of the premature bark and the increasing girth of the lower stem eventually results in the appearance of cracks in older plants. Symptoms may also occur eventually in the epicotyl, above the cotyledonary node. Inoculated plants, observed over 2 years, continue normal growth and can be separated from adjacent control plants only by bark disorders in the lower stem.

The results of the inoculation test using different cacao cultivars are presented in Table 8. Sample plants were removed at monthly intervals for internal examination and isolation. Preliminary isolations on agar were unsuccessful, probably due to fluctuating laboratory temperatures and rapid desiccation. Most samples required a long incubation period for colony establishment and for this reason PDA deeply poured in test-tubes was employed and maintained at 24°C.

Moist, brown-black, scattered to aggregated, irregular lesions occur in the cortical tissues. This necrosis is generally superficial but cankers may develop at

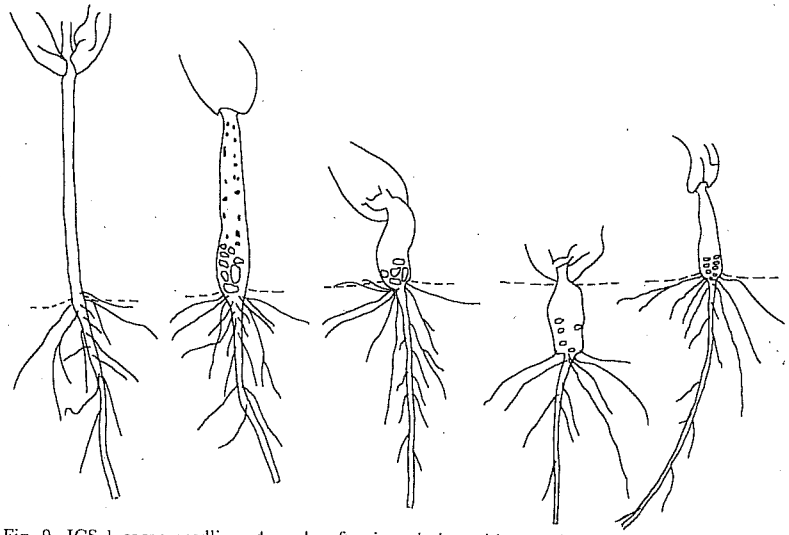


Fig. 9. ICS-1 cacao seedlings 4 weeks after inoculation with a conidial suspension of *M. roreri*. Control on left. Note stunted swollen stems and irregular pustules around stem base.

the stem with invasion of the wood and pith. In older plants the lesions have a dry flaky appearance. Discoloration of the cortex may extend up to 15 cm above the cotyledonary node without external symptoms. ICS-1 appears to be particularly susceptible to this systemic invasion of the upper stem. Microscopically the darker lesions consist of groups of heavily tylosed cells and mycelium is never seen. However, in the lighter coloured lesions, swollen (4-7 μm) convoluted hyphae pass intercellularly between the parenchyma cells, in the same manner and with the identical appearance of the mycelium in living tissues colonized by *C. perniciosa*. The mycelium is never present in quantity and is probably in an active state only at the edges of the fresh lesions, which may further explain the difficulties involved in isolating the fungus from stem tissues and the low rate of success per seedling. Nevertheless it was re-isolated from plants inoculated 22 months previously (Table 8) and from all the cultivars tested, although it was never isolated from dead plants which yielded typical cacao saprophytic fungi usually associated with weakened tissues, viz. *Botryodiplodia theobromae, Fusarium decemcellulare* Brick (*Calonectria rigidiuscula* (Berk. & Br.) Sacc.) and *Pestalotia* sp.

b) *T. angustifolium*

Symptoms become apparent towards the end of the second month. The hypocotyl is invariably swollen above and below soil level, particularly around the node, and bark abnormalities extend into the epicotyl. The latter is frequently stunted and noticeably lumpy or rippled. Root systems are not affected. Three to four months after inoculation badly stunted plants become necrotic and die, the tissues remaining rigid and mummified. Internally the hypocotyl is usually heavily cankered and invaded by saprophytic fungi.

c) *T. bicolor*

This species is highly susceptible to seedling infection and may be useful as an indicator plant to test the pathogenicity of *M. roreri* isolates.

One month after inoculation most plants are indistinguishable from the controls, although several may be stunted due to failure of the hypocotyls to

Table 8. Results of seedling inoculations with *M roreri*.

Host plant	Symptoms			Isolation of the fungus		
	Bark abnormalities	Stunting or stem swelling	Deaths	Max. time after inoculation (months)	Time on agar (days)** Average	Range
T. cacao cultivar						
Catongo	26/30*	8/30	5/30	8	28	21-60
Nacional	24/25	7/25	6/25	5+	16	7-28
EET-19	28/40	7/40	0/40	13	22	16-42
EET-233	45/60	8/60	6/60	19	17	10-61
ICS-1	46/55	12/55	8/55	20	23	14-60
Local hybrid	27/28	12/28	0/28	22	18	7-42
T. angustifolium	15/15	9/15	15/15	—	—	—
T. bicolor	66/70	64/70	61/70	8+	14	7-65
T. mammosum	34/35	27/35	22/35	9+	18	12-50

* Figures represent number of plants with symptoms /number inoculated.
** Until establishment of colony.
\+ At termination of experiment.

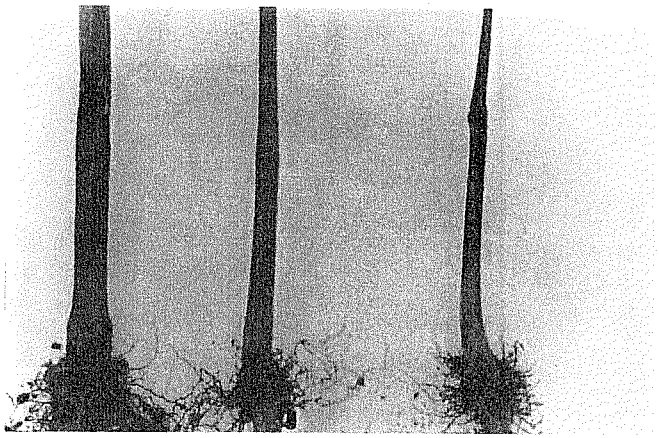


Fig. 10. Seedlings of *Theobroma bicolor* 5 months after inoculation. Control in centre.

elongate. Thereafter differences between the treated and control plants increase, with the former developing stem swellings or distortions and premature bark formation (Fig. 10). In some replicates, however, 3-4 months can elapse before these symptoms develop. There is a progressive decrease in growth rate, accentuated by leaf chlorosis in 4- to 5-month-old plants. White pustules, blisters and abnormal lenticels appear below and above the cotyledonary node and stem rippling frequently extends to just below the growing tip. Cankering, blackening and drying-out of the lower stem later leads to ring-barking and death. Leaves and stems wither but do not rot and there is a gradual mummification of the whole plant. The root systems show no abnormalities and are comparable with those of the controls. Deaths occur 2-3 months after inoculation but most commonly in 5- to 6-month-old plants. Few plants with external symptoms survive longer than 8 months.

Internal symptoms are equally severe, with red-black cankers around the stem base and often spreading into the whole of the lower stem. Lesions are generally more scattered and lighter in colour above the node, invariably confined to the cortex. The swollen intercellular mycelium is relatively abundant in the tissues and isolation from them has a high rate of success.

d) *T. mammosum*

Early external symptoms of infection are difficult to observe due to the hairy stem. However, it is not uncommon to find that the epicotyl is swollen or distorted and the plumule does not emerge. Plants with this condition shrivel and wilt when 5-7 weeks old. Others remain small and stunted compared with the controls. The upper and lower stems of the severely infected plants are noticeably hard and woody, and these eventually mummify 3-4 months after inoculation. At 4-7 months, most of the inoculated plants show symptoms in both the hypocotyl and epicotyl: discoloured thick bark often flaking and cracking; swellings or ripples; stimulation of the axillary buds and subsequent death of the lateral branches; thickening or swelling of the leaf base.

All the surviving plants, removed and examined 9 months after inoculation, revealed necrosis throughout the cortex and fresh brown lesions extending some 20-25 cm above the cotyledonary node. The cotyledons frequently harden or mummify and persist.

Stem infection

1. Inoculation

In cocao plants the new vegetative growth or flush remains soft and pliable for some weeks. With time, the stems develop a thick cuticle and harden. Therefore two types of stem tissues—hardened and unhardened—were delimited for inoculation studies.

A spore paste, obtained from agar or barley grain cultures of *M. roreri*, was smoothed on to the stem surface near the growing tip with a spatula and immediately covered with a cotton-wool pad soaked in sterile distilled water. All inoculations were carried out in the greenhouse on 1- to 2-year-old cacao seedlings.

2. Symptoms

No symptoms were observed in any of the plants inoculated at the hardened stem stage. But, of the 45 plants inoculated at the light green or unhardened stem stage, 27 showed evidence of infection. In a few plants the first symptoms appeared after 3-4 weeks with the formation of cork cells or premature bark and slight swellings or ripples in the immediate inoculation zone. However, in the majority pronounced symptoms did not occur until *c.* 6 weeks after inoculation. Stem swellings, blisters and necrosis were commonly present, often extending into the soft tissues above. In plants which had flushed during this interval the newly formed flushes were sometimes distorted, abnormally rigid and with black blisters, premature bark and abnormal lenticel formation. Presumably the fungal mycelium had penetrated the meristem and grown with the flush tissues causing unequal swelling and subsequent distortion of the whole stem. Advanced symptoms, 8-12 weeks after inoculation, were cracking of the stem in the region of premature bark formation and a slow spread of necrosis or cankering. Black, water-soaked lesions occurred internally in the cortex and occasionally in the rays of the outer wood. The blackening of the cortex is due to excessive tylose formation or cell occlusion and mycelium is difficult to observe and isolate in culture.

Cushion infection

1. Field observations

The first suggestion that the pathogen may invade flower cushions via infected pods and later reinfect pods formed on that cushion or on neighbouring cushions was obtained from extensive pollination studies done during the long dry season at Pichilingue (D. F. Edwards, personal communication). Young hybrid trees bearing maturing pods consistently showed high pod losses due to frosty pod rot and the pods on any one tree often showed lesions within several days of each other, but without previous external symptoms. As the pods had developed only in the dry season and all the trees had been rigorously cleaned to remove possible inoculum sources, it was postulated that the fungus had a systemic phase within the pod cushion or adjacent stem.

Further evidence to support this theory came from internal examinations of newly infected pods and the bearing cushions. Pink to light brown discoloration of the vascular rays occurred in the peduncle and outer wood of the stem, sometimes leading to the formation of a superficial canker in the trunk below the cushion. In thin branches, the discoloured vascular rays could be traced back to the main stem. However, despite numerous attempts, *M. roreri* was never isolated in pure culture. Similar branch cankers were also found in heavily diseased *T. angustifolium* trees and *M. roreri* was sometimes isolated from the discoloured woody tissues. Earlier, Naundorf (1955) had noted that the peduncles of diseased fruits had black vascular tissues which extended as far back as the cortex, cambium and adjacent wood of the tree. He suspected that

the fungus invaded the tissues of the cushion and reinfected pods borne on that cushion. He failed, however, to confirm this hypothesis experimentally.

2. Field investigations

A plot of 150 cacao trees of mixed genetic origin was selected in a farm near Pichilingue. The trees were of indeterminate age but most had evidence of heavy cropping over some years. All the flowers appearing on the trunks were pollinated up to hand height from October to November of the 1975 dry season. In half the trees the pollinated flowers were immediately enclosed in small plastic bags. The flowers on the remaining trees were left unprotected. The bags were replaced with larger ones, sealed with expandable labels, as the pods increased in size. The value of using plastic bags throughout the growth of a pod to reduce losses due to *M. roreri* had been proved in previous inoculation studies. Recordings of diseased pods were made weekly until July 1976 and identification of the causal organisms was confirmed by laboratory examination and isolation where necessary.

The results are presented in Table 9, and they demonstrate the effectiveness of plastic bags in reducing losses due to *M. roreri* and *C. perniciosa*. However, in waterborne diseases such as *Phytophthora palmivora* little protection is given. During the 1976 season pod losses due to *C. perniciosa* were far higher than those due to *M. roreri*, emphasizing the importance of the former fungus as a pod pathogen. In the absence of sporulation, pods infected with *M. roreri* were indistinguishable from those with *C. perniciosa*.

The unbagged pods infected with *M. roreri* varied in age from 3 to 6 months and a large proportion had prior symptoms in the form of swellings. All the bagged pods with the disease were mature to over-ripe, 5-7 months old, without swellings, with dry internal rots and on old cushions. Most appeared within a short period, towards the end of March, and cushion dissection revealed the presence of vascular ray discoloration. One pod was associated with a superficial canker in the outer wood of the trunk. Isolations were made from all cushions and *M. roreri* was obtained in culture from one cushion only.

Table 9. Analysis of disease losses in bagged and unbagged pods in a cacao farm near Pichilingue.

Treatment	Number of diseased pods		
	M. roreri	*C. perniciosa*	*P. palmivora*
Without plastic bags	56 (18·0)*	102 (32·9)	48 (15·6)
With plastic bags	8 (2·4)	6 (1·8)	57 (17·3)

* Figures in parentheses are percentage infection of total crop.

This type of experiment provides circumstantial evidence that *M. roreri* invades the pod cushion, remains systemically within it and may later infect pods borne on that cushion. The ability of the fungus to invade and persist in cacao tissues, other than pods, was also demonstrated in the previous two sections. The stimulus to grow from the cushion into the pod may be the result of changes in cushion sugar levels as the pods approach maturity. The percentage of pods infected directly from the cushion is probably low but infected cushions may constitute an important source of seasonal carry-over.

More proof is still needed to support this indirect evidence in view of the difficulties in isolating *M. roreri* from woody tissues; perhaps by using cushions with a known history of infection. Furthermore, it is possible that intracellular invasion of the cushion tissues by systemic mycelium of *M. roreri* may provoke cherelle wilt without actual invasion of the pod.

VI THE DISEASE CYCLE

Effect of climate or season

Climate has been considered to some extent in Section IV, but here it is applied specifically to its effect on the disease cycle. The experiments reported below have been designed to elucidate the life cycle of *M. roreri* under the climatic conditions existing in western Ecuador, with particular reference to the Arriba zone where the bulk of the Ecuadorian cacao is cultivated. This area has an unusual climate for cacao: a wet season from mid-December to June or July, with abundant rainfall and relatively high temperatures; a dry season in the remaining months, characterized by lower temperatures, an almost continuous cloud cover and occasional mists which prevent excessive evapo-transpiration. The greater proportion of the crop is harvested at the end of the wet season and the number of pods developing during the dry season is small. Thus a survival problem exists during the dry months in view of the lack of both host material and favourable conditions for infection. In other cacao-growing zones in Ecuador and Colombia the rainfall tends to be more evenly distributed and suitable conditions for infection may persist throughout the year; however, there is still an inter-crop period to be considered. The critical factor in the disease cycle in western Ecuador is the marked dry season and it is important to determine how the fungus survives between crops and the sources of inoculum available at the beginning of the wet season.

Conidia

The asexual spores of *M. roreri* are the only direct infective propagules known. They are produced in great abundance on diseased pods and have not been detected on any other cacao tissues in the field.

1. Dispersal

Pentatomid stink bugs, in addition to providing entry sites for *M. roreri* on the pod, were also considered by Naundorf (1954) to be carriers. Conidia were found on the body parts of these insects, and also on caterpillars, but more complete proof of their role as carriers is lacking.

Pods infected with *M. roreri* do not attract a large or specific insect fauna, in contrast with those infected with *P. palmivora*. Furthermore, pentatomids feed only on the healthy parts of pods and in western Ecuador are common only during the dry months of the year. Their role as carriers, in Ecuador at least, must be regarded as minimal, as also must that of other insects. From the beginning, Rorer (1918) had considered the possible role of insects in dissemination and penetration, notably by membracids, but he concluded that they are not necessary for infection.

The sporulation density on the pod is such that by merely blowing on the surface, clouds of conidia are liberated and freely carried by the wind or prevailing convection currents. It has been calculated that the sporulation density can reach 44 million conidia per cm^2 and that a mature pod may produce over 7000 million conidia (Campuzano, 1976). Even on mummified pods, more than one year after infection, conidia were collected when air was blown over the surface (Fig. 11). Water, as raindrops or drip, is effective in releasing small clouds of spores. The dry conidial masses are thus efficiently dispersed into the air by any physical contact with the infected pod. Strong winds are infrequent in the cacao-growing region of Ecuador but light prevailing winds occur intermittently during the wet season. The presence of convection currents in the cacao farms—following daily fluctuations in temperature—will further disperse conidia within the farm.

That *M. roreri* is a common component of the air-spora at Pichilingue has been determined by regular collections of the conidia using a Hirst volumetric spore trap, situated *c.* 1 km from the nearest cacao plantation. Conidia were

Fig. 11. Infected mummified cacao pods after almost one year on the tree. Relatively few conidia remain on the persistent white pseudostroma.

identified using reference slides, previously exposed near an inoculum source. Preliminary results indicate that conidia occur throughout the year with a high frequency from 11.00-18.00 hours during the wet season, possibly resulting from increased wind velocity prior to the afternoon rains, and with more scattered distribution during the dry months. In December 1974, at the start of the rains, there was a marked increase in the number of conidia deposited and most were globose, brown, thick-walled and large (10-12 μm). It is suggested that these represent old conidia released from mummified pods by the physical action of the rain. A low percentage of the conidia germinated *in situ*.

2. Survival

Information on the viability of the conidia of *M. roreri* is minimal and contradictory. Naundorf (1954) and López (1954) used laboratory germination as a measure of viability and concluded that it was only 1-10 days. The former author also inferred that this would be further shortened by a dry environment. Wellman (1972) states that the conidia are readily killed by sun, drought and cold. However, Cabanilla (1966) stored dry and freeze-dried conidia in Petri

dishes and tested their viability or infectivity by inoculation of pods in the field. With both treatments high infection rates were obtained up to the end of the experiment 315 days later. All workers (Naundorf, 1954; López, 1954; Bejarano, 1961; Author) found extremely low levels of germination in laboratory tests, especially with 'older' spores (> 10 days). It would seem that there is a dormancy mechanism which may be linked with thickening of the spore wall. Germination cannot be used, therefore, as a test of viability until more is known about the factors controlling it.

A series of experiments was established at Pichilingue, designed to test the viability of conidia from all potential inoculum sources available in a cacao farm, using a pod inoculation technique.

a) In the soil

There are two possible forms of spore survival in the soil—directly in or on the soil as wind-blown or rain-dispersed conidia, and on diseased pods left after harvesting on the soil surface.

Large plastic pots were filled with soil from the upper horizon in a cacao farm and stored on trestles in an isolated palm frond shelter. In half the pots (treatment 1) the soil was liberally dusted with conidia from freshly sporulating pods. In the remaining pots (treatment 2) 1 cm^2 pod pieces, taken from a newly sporulating lesion, were placed on the soil surface. All pots were lightly covered with cacao leaf litter. The experiment was begun in September 1973 (during the dry season) and sufficient replicates were included to allow for sampling over 2 years. At monthly intervals four pots were selected from each treatment and an inoculum suspension was prepared. In treatment 1, the conidia, visible as a cream powder, plus surface soil were mixed into 400 ml of sterile distilled water. For treatment 2, the surface of the pod piece was lightly scraped with a spatula into water, as above. After thorough agitation 4 ml aliquots were taken from the suspensions and pipetted on to moist cotton-wool pads. The latter were then used to inoculate 30- to 40-day-old, hand-pollinated pods. In the wet season the pods were protected from external contamination with plastic bags. In addition to an equal number of control pods, a third treatment was included in which 'fresh' conidia were inoculated in suspension. The trees used in the study were of mixed genetic origin and all three treatments plus the control were compared within any one tree. Pods were observed until maturity, and the time to lesion appearance and sporulation was noted.

Results are summarized in Table 10. Conidia in the soil showed low infectivity after 1 month and none at 3 months. Conidia on pods in the soil were still highly infective at the 1 month stage but infectivity decreased sharply with 2- to 3-month-old conidia and no infections resulted thereafter. The experiment was ended after 8 months when it was considered that the absence of infection was consistent; using fresh conidia, 90% or more infection was obtained. During the preparation of conidial suspensions for treatments 1 and 2, samples were examined microscopically and found to consist predominantly of empty conidial sacs. Bacteria and nematodes were observed inside newly lysed conidia and it is suggested that the activities of the soil fauna and flora caused the destruction of the conidia, thus reducing the period of infectivity of this inoculum source to a few months. The fact that the spores were in the soil during the dry season may also have contributed to the loss of infectivity but it is thought that desiccation *per se* is not a critical factor, as shown by the results of Cabanilla (1966).

b) On hanging pods

Pods infected with *M. roreri*, especially those infected at 2-6 weeks, usually dry rapidly and mummify on the trees, remaining attached for a long time (Fig. 11). Conidia of known age, taken from hanging pods of treatment 3 in the previous experiment, were used to provide an inoculum suspension. In addition, it has

Table 10. Survival of conidia of *M. roreri* in cacao soil and on pods on the soil as tested by pod infectivity.

Treatments	Age of conidia in months								Time to lesion (days)	
	1	2	3	4	5	6	7	8	Mean	Range
1. Conidia in soil	32*	14	0	0	0	0	0	0	72 ± 3	53-83
2. Conidia on pods in soil	87	21	6	0	0	0	0	0	66 ± 5	48-82
3. Fresh conidia	96	93	96	94	91	92	93	90	60 ± 6	47-83

* Number of pods infected after deduction of diseased control pods.
100 pods per treatment inoculated at 30-40 days of age.

been observed that infected pods frequently produce numerous conidia internally on the inner pod wall and on the bean mass. Since these conidia are less effected by the external environment than those produced externally, they were also tested for infectivity. Experimental details are as described above except that all pods were enclosed in plastic bags from pollination to maturity.

A range of cacao cultivars was used but, because of the number of treatments involved, it was not possible to compare the treatments within any one tree. From the results (Table 11), there appears to be no evidence that wilt is associated with conidial infection of cherelles. Infectivity was consistently high with conidia up to 7 months of age but this then decreased sharply and no infected pods were obtained with 10- or 12-month-old conidia. The spores from the interior of mummified pods showed a similar infectivity pattern, although 9-month-old conidia still remained highly infective (see Fig. 4).

Far less empty conidia were observed compared with the soil treatments and even with conidia 7 months of age, the majority were fully turgid and intact. It is concluded that the low level of biological degradation on hanging pods, probably connected with rapid drying of the pod tissues especially in those pods infected at the end of the rainy season, ensures that a high proportion of the conidia survive or retain viability for prolonged periods, sufficient to provide an efficient means of seasonal or intercrop carry-over.

Table 11. Survival of conidia of *M. roreri* on mummified hanging pods as tested by pod infectivity.

Age of conidia in months	Number of pods infected	Days to symptoms		
		Swelling	Lesion	
			Mean	Range
From pod exterior				
1	85 (7)*	35-55 (44·7)**	69 ± 10	45-90
2	81 (15)	45-55 (96·3)	60 ± 4	55-72
3	95 (4)	50-55 (100)	59 ± 5	51-79
4	88 (7)	50-55 (100)	62 ± 5	49-74
5	77 (16)	40-55 (97·4)	59 ± 7	51-90
6	87 (12)	40-55 (85·0)	54 ± 3	51-66
7	82 (4)	40-65 (89·0)	58 ± 6	45-89
8	52 (23)	44-65 (100)	62 ± 7	47-83
9	14 (20)	44-50 (20·9)	59 ± 3	48-81
10	0 (18)	—	—	—
12	0 (12)	—	—	—
From pod interior				
3	79 (9)	50-55 (89·9)	64 ± 5	51-89
9	58 (13)	50-60 (84·5)	70 ± 3	51-90
12	0 (19)	—	—	—
Control	0 (18)	—	—	—

* Figures in parentheses are numbers of wilted pods.
** Figures in parentheses are % infected pods with swellings.
100 pods per treatment.

Mycelium

1. Saprophytic

Early reports from Ecuador described a fungus similar to *M. roreri* that grew on the soil surface under cacao trees (Anon., 1953a; Orellana, 1954). Naundorf (1954) was also of the opinion that the pathogen had a distinct soil phase. This later led to the testing of eradicant fungicides on the soil to reduce disease losses (Anon., 1960). The following is translated from the last paper: "The rapid increase in pod rot early in the rainy season has been considered to be due to the ability of the fungus to exist and multiply in the upper soil and debris during the dry season".

Nevertheless, there are no further reports along these lines and examination of cacao soils and litter, both in the field and in pots seeded with conidia, has not revealed the presence of a free-living saprophytic stage. In the Quevedo area a fungus easily confused macroscopically with *M. roreri* grows directly on cacao soil, often associated with earthworm casts, at the end of the rainy season and persists into the dry season. The soil colony consists of a white periphery and a central tan-coloured sporulating area producing a crust of conidia, strikingly reminiscent of *M. roreri*. This fungus is a species of *Ostracoderma (Chromelosporium)* and it is reasonable to assume that it is the same fungus previously referred to as the saprophytic soil phase of *M. roreri*.

2. Systemic or parasitic

The systemic invasion of the cushion or stem tissues and the ability to remain within them for prolonged periods, as shown by isolation of the fungus from inoculated seedlings, constitutes an efficient mechanism for survival during periods unfavourable to continued activity. If, as suggested earlier, the pathogen invades mainly maturing pods from the cushion via the peduncle this potential source of inoculum will not assume importance until later in the cropping season. The systemic pod mycelium constitutes the prolonged biotrophic phase of the life cycle which, due to the absence of degradative enzymes, permits continued host growth. Thus the pod tissues are intimately colonized prior to necrosis resulting in extremely rapid and abundant sporulation during the relatively short necrotrophic phase.

Sexual stage

The existence of a sexual stage in the disease cycle seems doubtful. Mummified cacao pods, infected branches and inoculated dead seedlings have been stored in a humid gauzehouse and observed over 2 years but no related fruiting body has been identified. Similarly, a range of macrofungi, typically found in local cacao farms, has been screened by spore isolation on agar without success. The discovery of a sexual stage would be of special interest as the taxonomic evidence shows that *M. roreri* is the asexual form of a basidiomycete and there are enough similarities to speculate that it may be closely related to *C. perniciosa*.

Summary

The cycle summarized below is applicable to the conditions existing within a cacao farm in western Ecuador, specifically when an extended dry period is involved. However, it can be modified or interpreted to cover local variations in environment.

1. Initiation of the disease cycle at the beginning of the rainy season, December-January, from primary inoculum sources, viz. conidia on hanging pods. Presence of free water allows germination of conidia and also removes inoculum from mummified pods in the canopy. Some diseased pods, systemically infected from the cushion, may appear but this depends on the cropping pattern. Flowering and fruit setting are increasing during this period.

2. Pods infected by conidia in December-January will not sporulate until February-March, i.e. a 2-month lag period, when there will be dispersal by wind, convection currents or rain, coinciding with the time of major pod production. Pods infected from systemic mycelium in the cushion may now constitute an important primary source of inoculum.

3. After the 2-month time lag, pods infected in February-March sporulate, this inoculum contributing to the maximum recorded disease losses from June-August.

4. Pods infected towards the end of this period (July-August) may be left on the tree, as they show no symptoms and are still unripe when the harvesting tails off in September. These will sporulate during the dry season and retain viable conidia for up to 9 months, sufficient to provide an adequate inoculum at the onset of the rains.

5. Trees with a continual or irregular cropping pattern may harbour infected but symptomless pods during dry or inter-crop periods and are thus potential sources of primary inoculum when the rain or normal pod setting begins.

6. Any infected pod left on the tree to mummify from April onwards will constitute an important source of seasonal carry-over to initiate the disease cycle at the beginning of the rains in December.

VII CONTROL

Crop sanitation

It is evident from the proposed disease cycle that good crop sanitation should effectively reduce levels of inoculum, particularly if there is a well defined inter-crop or dry period. It is recommended in the latter case that at the final harvest all infected pods are removed from the tree and left on the soil surface. Removal of diseased but non-sporulating pods from the tree by regular harvesting will reduce inoculum sources on the tree and may also limit systemic invasion of the cushion. But frequent harvesting often does not suit the farming patterns of the smallholder. Newly diseased pods will sporulate on the soil surface and as such will contribute to local infection during the wet season but are unlikely to be important sources for more than 1-2 months.

None of these proposals is new and crop sanitation has long been stressed for the control of frosty pod rot. Rorer (1918) recommended improving drainage, light pruning and the destruction of diseased pods. In heavily infected plantations, however, he noted that the handling of diseased pods may only create more infections. Jorgensen (1970) supports this conclusion from field experience, as losses dramatically increased when this form of crop sanitation was practised. The diseased pods were not dipped into any fungicide and greater dissemination of the spores resulted. Lass (1970) reported that a large hacienda in Ecuador collected pods infected with *M. roreri* before the main harvest and dipped them in agricultural oil. Subsequent disease losses were low. The beneficial effects of removing diseased pods from the trees has been shown in large scale field trials in Ecuador (Desrosiers, 1960; Desrosiers & Suárez, 1974). These examples would seem to confirm the importance of hanging, mummified pods in initiating the disease cycle. It is now considered that harvesting the diseased pods about 3 months before the start of the rainy season, without further treatment, would greatly reduce the primary inoculum sources and, in effect, delay the build-up of an epiphytotic. The burial of diseased pods before sporulation, initially recommended by Rorer (1918), has often been suggested but Jorgensen (1970) has pointed out the practical difficulties involved, particularly in view of the short interval between lesion appearance and sporulation. Early identification of the disease at the swollen or distorted stage—long before sporulation—would be beneficial in this form of control.

The value of cultural practices for the control of *M. roreri* has been extensively investigated in Colombia. Barros (1966) tested a variety of crop sanitation methods, including regular harvesting and pruning. Treated plots showed increased yields amounting to *c.* 460 kg of dry cacao per hectare, and this was attributed to the reduction in *M. roreri* pod rot following crop sanitation. The

latest recommendations by the Colombian extension service involve mainly crop sanitation for disease control (Barros, 1975b). Strong emphasis is placed on lowering the relative humidity of the cacao plantation by reducing shade and regular pruning. Weekly or bi-weekly harvesting with the removal and burial of all pods with visible symptoms is advised. Whereas reduction in shade and thinning of the cacao canopy may reduce humidity it may also improve air circulation and so favour spore dispersal. The thinning of cacao plantations has also been proposed in Ecuador (Anon., 1953b). Earlier Rorer (1918) had advocated planting at a high density to increase production, as he found no correlation between close planting and the amount of frosty pod rot. Trials are now in progress in Colombia to determine the effect of planting distances on disease losses due to *M. roreri*.

Spraying

Rorer (1918, 1926), after several years of spraying trials with copper and sulphur compounds, considered that this form of control was uneconomic. He concluded that . . . . 'these experiments (fungicide) showed that many applications at frequent intervals were necessary to control the disease at all successfully and that the cost of the work was absolutely prohibitive'. Naundorf (1954) and Garcia (1954) also reported disappointing results with fungicides in Colombia, although soil spraying was found to reduce disease incidence. Observations in Ecuador by Jorgensen (1970) suggest that the disease cannot be controlled by the application of fungicides to the soil.

Desrosiers & Diaz (1956) and Desrosiers (1957) reported reductions in losses due to frosty pod rot and spectacular increases in yield following spraying with sulphur, copper and zinc based fungicides. The experiments were conducted in an abnormally dry year and according to Jorgensen (1970) this is the only fungicide trial which has given good results. He concludes from his experiences on a large commercial cacao plantation in Ecuador, which conducted extensive spray trials over some years. that it does not pay to control the disease by spraying in old plantations, and it is doubtful whether it is economic in young plantations. He also cites a number of unpublished spraying experiments in support of these conclusions (see Table 12).

Recent work in Ecuador has involved the use of systemic fungicides. Sotomayor (1973) tested a range of systemic fungicides applied to either the soil, the pods or the foliage of trees bearing pods artificially inoculated with *M. roreri*. The results were, however, erratic and at low or high dosages there was no evidence of effective control. In Venezuela Meza Sanoja (1973) screened several fungicides and found that with Benlate yields increased with rates of application, although 3 years' data from a field trial in Ecuador showed that losses due to *M. roreri* in the Benlate sprayed plots were higher than those in the control plots (Anon., 1971). The failure of Benlate to act against basidiomycete fungi, combined with the possible enhancement of spore dispersal following motorized spraying, may account for these results. Spraying trials to test new fungicides or combinations of fungicides and cultural practices are still in progress in both Ecuador and Colombia. However, in advocating spraying as a form of control, consideration should be given to the local system of cacao cultivation. The big plantations have virtually disappeared in Ecuador and production is mostly in the hands of the smallholder. In this system cacao is a low-input crop and the cost plus the complexities of accurate timing of spraying make the operation impractical. Even frequent harvesting may be uneconomic or alien to the accepted method of cultivation. It would seem more appropriate to simplify, as far as possible, the recommendations for control of the disease. A single thorough harvesting during the dry season or inter-crop period, to rid the tree of all mummified pods, may be the only present answer to effective control.

Table 12. Effect of pruning and/or fungicides on incidence of *Moniliophthora* pod rot, Hacienda San Antonio, Ecuador (from Anon., 1971).

Treatments	*Moniliophthora* incidence			Production data	
	Pods totally destroyed	Pods partially destroyed	Percentage infection	Healthy harvested pods	Wet bean weight (gm)
1. Fungicide spray (Manzate, 2 lbs a.i./acre)	1514	163	23·3	5525	686,495
2. Fungicide + pruning	1164	221	20·6	5333	684,463
3. Pruning	1658	226	26·1	5333	679,231
4. Control	1998	147	27·8	5576	686,841

Resistance

Rorer (1918, 1926) made repeated references to the low disease incidence observed in Nacional cacao plantations: 'During 1918, which was a very wet year, the disease spread very rapidly, and even attacked Nacional to some extent'; 'This variety of cacao (Nacional) apparently has a great amount of natural resistance . . . .'. Similarly the Criollo or native cacao of Colombia has been reported to suffer only slight losses (Naundorf, 1954; Wood, 1959). There is some suggestion that these indigenous cultivars have had a long association with *M. roreri* and consequently may have developed some immunity.

Delgado *et al.* (1960) studied the losses due to frosty pod rot in a number of clones over 3 years in Ecuador, and concluded that there were wide differences in the amounts of infection but these were not necessarily related to the genetic origins of the clones. As suggested by Thorold (1975), cropping behaviour rather than resistance may have accounted for their results. Later Sotomayor (1965) developed a pod inoculation technique to test these clones, but all the inoculated material proved to be susceptible and it was considered that the inoculum level employed was too high. Using lower spore concentrations, part of the cacao germplasm collection at Pichilingue has been screened for pod resistance to *M. roreri*. The results have demonstrated that most clones are highly susceptible (Anon., 1971). However, clone EET-233, a Nacional × unknown hybrid, showed only low disease incidence and on the infected pods lesion development was restricted. Further study of this material and, indeed, of Nacional is warranted. Recent pod inoculation studies carried out by the author have confirmed the observation that lesion formation and sporulation are inhibited in certain hybrids (particularly involving those with Nacional) and that although the pods are susceptible to infection *per se* a low sporulation density would, of course, be advantageous.

The popular belief that there is a correlation between disease incidence and pod pigmentation was dispelled by Diaz (1957) when he compared natural disease losses due to *M. roreri* in trees bearing yellow or red pods.

Crop manipulation

In trees which produce the bulk of their crop during the drier months of the year, there is evidence to suggest that disease losses are consistently low (D. F. Edwards, personal communication). This disease escape, through atypical cropping pattern, is a form of indirect control.

The possibility of artificially manipulating the cropping pattern in existing cacao is now under investigation in Ecuador. By keeping trees free from pods until the start of the dry season and by controlled stem wounding, sufficient flowers are available to allow for subsequent hand pollinations during the dry months. Maturation occurs during the first months of the wet season but the pods reach maturity and are harvested before the infection cycle is completed. These experiments are providing encouraging results both by improving pod set and by reducing disease losses (Evans *et al.*, 1977).

As previously pointed out, the continuous cropping trees are potentially dangerous sources of inoculum carry-over. Cacao cultivars with short cropping periods, e.g. Nacional, may escape heavy disease losses as they will tend to carry fewer sources of primary inoculum at the beginning of the rainy season. Build-up of infection will thus be slow and irregular.

VIII COMPARISON WITH *CRINIPELLIS PERNICIOSA*

Crinipellis perniciosa, the cause of witches' broom disease of cacao, is thought to have its centre of origin in the upper Amazon basin, where it co-existed with wild cacao or other *Theobroma* species (Baker & Holliday, 1957). Its spread to western Ecuador was probably aided by man (Pound, 1938). However, a strain of *C. perniciosa* has recently been reported on lianes and various trees in forested

areas of western Ecuador, suggesting that the fungus is endemic to this area (Evans, 1978). This strain, essentially non-pathogenic or saprophytic, can invade *Theobroma* tissues but severe symptoms, in particular the characteristic formation of brooms, are absent. Frosty pod rot and witches' broom occur in all the cacao-growing regions of western Ecuador and are the principal factors limiting production. Their individual effects on yield are difficult to assess since the diagnostic characters used to differentiate between both types of pod infection are at variance. Comparative pod inoculation experiments, made recently at Pichilingue, have show that infected pods, at all stages of development, present identical symptoms both externally and internally. They can, of course, be easily distinguished when *M. roreri* sporulates on the pod surface. Nevertheless, with regular harvesting, as in field trials to determine pod disease losses, the infected pods may be removed prior to sporulation and are then classified according to poorly defined symptoms. Accurate identification of the causal fungus can be made only by cutting the pod open and examining the resultant mycelium several days later. The fact that both fungi produce similar pod symptoms, which in themselves are unusual, led the author to consider their origins and relationships and to compare their characteristics.

The resultant detailed analysis of conidiogenesis and hyphal ultrastructure has elucidated the true taxonomic position of *M. roreri* and strengthened the hypothesis of a common link between these two endemic South American cacao pathogens. Although *M. roreri* can infect cacao flush tissues at high spore concentrations and induces symptoms essentially similar to those caused by certain strains of *C. perniciosa* on highly resistant cacao cultivars, it does not grossly overactivate the meristems to form the abnormal shoots or witches' brooms so characteristic of virulent strains of *C. perniciosa*. Nevertheless, *M. roreri* does provoke hypertrophy and hyperplasia in cacao pods and therefore, in common with *C. perniciosa,* has the ability to disrupt the hormonal balance of the host. The intercellular mycelium of both fungi is typically swollen and convoluted, being distinguished from the intracellular mycelium and from that in standard culture. This primary or biotrophic mycelium of *C. perniciosa* can now be cultured on cacao callus medium (Evans, 1980), and this will enable a closer examination to be made of the parasitic behaviour of both fungi.

Until more evidence is available, it is tentatively suggested that *M. roreri* and *C. perniciosa* have a common origin, both forms having evolved as highly pathogenic races from the original saprophytic or weakly parasitic liana strain: *M. roreri* in the foothills of the western Andes and *C. perniciosa* in the Amazon basin. *C. perniciosa* is basically a fungus of the lowland tropics and, until very recently, had not been reported from higher altitudes, whereas *M. roreri* is widespread in the elevated cacao-growing regions of Colombia. It is possible that basidiocarp formation in *C. perniciosa* is retarded at temperatures below 20°C and that an arthroconidial-producing strain evolved in the Andean cordillera. Whether or not this controversial theory can ever be satisfactorily proven is doubtful, but it may be possible to gather circumstantial evidence for or against, by the use of serological tests. At the very least it may promote further and more intensive research on *M. roreri*, until recently a neglected and little known fungus.

IX SUMMARY

Moniliophthora roreri (Cif.) Evans *et al.* (1978), the causal agent of frosty pod rot disease of cacao, was first investigated in 1917 in the Quevedo area of western Ecuador, where it caused a dramatic reduction in yields in introduced Forastero cacao cultivars. The pathogen was ascribed to the form genus *Monilia* and later named in honour of J. B. Rorer who had pioneered its study. Earlier descriptions of cacao maladies indicate that the disease was present in

epiphytotic proportions during the 19th century in both Ecuador and Colombia.

The fungus attacks some *Theobroma* and *Herrania* species and it is likely that all members of these two New World genera are potential hosts. Its occurrence on wild *Herrania* and/or *Theobroma* species in Colombia and Ecuador suggests that it is indigenous to the north western part of South America. The pathogen is still confined to the countries of this geographic zone and to parts of Central America but poses a direct threat to the other cacao-growing countries of Central and South America as well as a potential threat to cacao cultivation in other parts of the world. The disease has not been found in the Amazon basin, although with the increasing agricultural development in this area chances of introduction of the fungus by man are high.

The climate range of *M. roreri* is wide; from sea level to over 1000 m, from low to high rainfall areas (40-1000 cm per annum). High rainfall is generally conducive to high disease incidence and the two are significantly related.

Average estimates of losses in Colombia and Ecuador vary from 20 to 40% of the total crop.

The conidia are the only known infective propagules and they penetrate directly through the epidermis of pods of any age. Symptoms, in the form of pronounced swellings, may appear within 30-50 days on pods infected at an early stage of development. External lesions occur 2-3 months after infection followed by sporulation 7 days or more later. The white, cream to brown powdery bloom on the pod surface is vividly described by the local Spanish names for the disease e.g. helada (frost), hielo (ice), polvo (powder).

The swollen conidiophores arise directly from a pseudostroma and conidia appear to form basipetally in chains. The conidia are variable in shape but predominantly globose, (6)8-10(12) µm diam., initially hyaline but becoming light brown and thick-walled with age. The presence of dolipore septa in the vegetative mycelium, indicating basidiomyetous affinities, and the development of the conidia in basipetal chains has necessitated the transfer of the fungus to a new genus, *Moniliophthora*.

The conidia are considered to be dispersed by wind and rain, with insects playing only a minor or incidental role. They are commonly present in the air spora of cacao farms throughout the year as shown by experiments using a Hirst volumetric spore trap. Germination of conidia may occur on the collecting slide in conditions of low temperatures (21-23°C) and high humidity (> 90%). However, germination under laboratory conditions is irregular and an in-built dormancy mechanism is suggested. Under field conditions, the conidia have prolonged viability on mummified hanging pods (up to 9 months) but those in the soil or on pods on the soil are invaded by soil organisms and their infectivity is limited to 2-3 months. The ability to resist desiccation may be due to chitin deposition in the spore wall.

There is evidence to indicate that the mycelium invades the pod cushion and may later systemically infect pods formed on that cushion or on neighbouring cushions. This is further supported by greenhouse inoculation studies which have demonstrated that, in addition to pods, the conidia can penetrate and colonize seedling tissues and unhardened flush stems, producing characteristic symptoms—premature bark, swellings or blisters, cankers. Slow systemic growth by an intercellular, swollen (4-7 µm), convoluted mycelium may occur without symptom expression. *Theobroma angustifolium, T. bicolor* and *T. mammosum* seedlings are very susceptible and death invariably results. *M. roreri* can be re-isolated from cacao seedlings up to 22 months after inoculation.

The infection of cacao flowers by conidia has not been confirmed and further work is needed in this direction. The most suitable form of control for the smallholder lies in good crop sanitation. The removal of all hanging pods in the inter-crop or dry season is strongly recommended as the simplest, most efficient and most economic treatment. Further removal of the harvested diseased pods

from the farm is not necessary. Control through the use of fungicides is of dubious value due to poor results and/or the economics of spraying. By eliminating the sources of primary inoculum—mummified hanging pods—before the period of major pod setting, it should be possible to delay significantly the onset of infection. In this respect, cacao cultivars with a restricted or short cropping period would be advantageous. Control through the use of resistant or less susceptible trees seems promising and there is evidence that the endemic cultivars of western Ecuador and Colombia offer some resistance to pod infection. Indirect control or disease escape by selecting cultivars which produce the bulk of their crop in the season most unfavourable to fungal infection, viz. the dry season in western Ecuador, is another line which should be pursued.

Finally, comparisons of disease symptoms show that *M. roreri* and *Crinipellis perniciosa* (Stahel) Singer, the causal agent of witches' broom disease of cacao, have similar characteristics. The theory is proposed that they represent two evolutionary branches of a single fungal species.

X. BIBLIOGRAPHY

Ampuero, E. (1967) Monilia pod rot of cocoa. *Cocoa Growers' Bulletin* **9**: 15-18.

Anon. (1953a) Posible existencia en forma saprofítica de *Monilia roreri*. *Informe Anual de la Estación Experimental Agrícola del Ecuador, 1951-52*: 27-28.

Anon. (1953b) Enfermedades del cacao en el Ecuador. *Boletín Servicio Cooperativo Inter Americano de Agricultura, Quito* No. 22.

Anon. (1960) Efecto de la aplicación de erradicantes al suelo en relación con los ciclos de aspersión de fungicidas para el control de la Monilia del cacao. *Informe Anual del Departmento de Fitopatología, Estación Experimental Tropical de Pichilingue, 1959* (mimeograph).

Anon. (1971) *Informe Anual Técnico, Instituto Nacional de Investigaciones Agropecuarias, Quito, Ecuador*: 348-352.

Anon. (1974) *Informe Anual Técnico, Instituto Nacional de Investigaciones Agropecuarias, Quito, Ecuador*: 122-128.

Aragundi, J. (1974) Evaluación de rendimientos e incidencia de enfermedades del cacao, en varias zonas ecológicas del Litoral Ecuatoriano. Ing. Agr. Thesis, University of Guayaquil, Ecuador.

Baker, R. E. D.; Cope, F. W.; Holliday, P.C.; Bartley, B. G.; Taylor, D. J. (1954) The Anglo-Colombian cacao-collecting expedition. *Report of Cacao Research, 1953*: 8-29. St. Augustine, Trinidad; Imperial College of Tropical Agriculture.

Baker, R. E. D.; Holliday, P. (1957) Witches' broom disease of cacao (*Marasmius perniciosus* Stahel). *Phytopathological Papers* No. 2, 42 pp.

Barros, O. (1961) El cultivo del cacao en algunas zonas productoras de Colombia. *Agricultura Tropical, Colombia* **17**: 523-530.

Barros, O. (1966) Valor de las prácticas culturales como método para reducir la incidencia de *Monilia* en plantaciones de cacao. *Agricultura Tropical, Colombia* **22**: 605-612.

Barros, O. (1975a) Influencia del pH en el crecimiento del hongo *Monilia roreri* Cif. & Par. *Noticias Fitopatológicas, Colombia* **4**: 78-88.

Barros, O. (1975b) Conozca la moniliasis del cacao. *Boletín de divulgación. Instituto Colombiano Agropecuario* No. 51, 15 pp.

Barros, O. (1977) Investigaciones sobre el hongo *Monilia roreri* Cif. & Par., causante de la pudrición acuosa de la mazorca del cacao: sus daños y su control. *El Cacaotero Colombiano* No. 3: 42-52.

Bastidas, A. (1953) Patogenicidad de *Monilia* sp. en *Theobroma cacao* L. *Cacao en Colombia* **2**: 139-154.

Bejarano, G. (1961) Métodos de inoculación artificial y factores favorables para la infección de *Monilia roreri* Cif. & Par. Ing. Agr. Thesis, Central University, Quito, Ecuador.

Burgos, J. A. (1954a) Algunos resultados del trabajo experimental sobre cacao en la Estación Experimental de Tingo María. *Report of the Inter-American Technical Committee on Cacao. Fifth Meeting, Turrialba, Costa Rica, Document* No. 27, 16 pp.

Burgos, J. A. (1954b) Comentarios y recomendaciones sobre el cultivo de cacao en las provincias de Jaen y Bagua, Perú. *Report of the Inter-American Technical Committee on Cacao. Fifth Meeting, Turrialba, Costa Rica, Document* No. 28, 8 pp.

Cabanilla, H. (1966) Effect of freeze-drying on viability of spores of *Monilia roreri* Cif. & Par. *Second Session FAO Technical Working Party on Cacao Production and Protection, Rome, Sept. 1966* (mimeograph).

Campuzano, H. (1976) Fluctuatión de poblaciones de esporas de *Monilia roreri* Cif. et Par. y viabilidad durante un ciclo completo de afección. *Noticias Fitopatolhgicas, Colombia* **5**: 107.

Chalmers, W. S. (1972a) UWI-INIAP Ecuador Co-operative programme. *Annual Report of Cacao Research 1971*: 25-27. St. Augustine, Trinidad; Regional Research Centre, Imperial College of Tropical Agriculture, University of West Indies.

Chalmers, W. S. (1972b) The conservation of wild cacao populations: the plant breeders most urgent task. *Report, Fourth International Cocoa Research Conference, Trinidad and Tobago, January 1972*: 114-118.

Chalmers, W. S. (1972c) INIAP-UWI expedition to collect *Theobroma cacao* in the Oriente region of Ecuador, June 25-August 10, 1972, 16 pp. (mimeograph).

Chapman, F. M. (1926) The distribution of bird-life in Ecuador. *Bulletin American Museum of Natural History* **55**.

Cheesman, E. E. (1944) Notes on the nomenclature, classification and possible relationships of cacao populations. *Tropical Agriculture, Trinidad* **21**: 144-159.

Ciferri, R.; Parodi, E. (1933) Descrizione del fungo che causa la 'Moniliasi' del cacao. *Phytopathologische Zeitschrift* **6**: 539-542.

Cooke, R. (1977) *The biology of symbiotic fungi*. 282 pp. London & New York: Wiley.

Delgado, J. C.; Ampuero, E.; Doak, K. D. (1960) Posible evidencia de resistencia a la *Monilia roreri* Cif. & Par. en algunos clones de la Estación Experimental Tropical de Pichilingue. *Report of the Inter-American Technical Committee on Cacao. Eighth Meeting. Trinidad and Tobago*: 184-192.

Desrosiers, R. (1957) Developments in the control of witches' broom. Monilia pod rot. Cerastomella disease of cacao. *Report of the Inter American Technical Committee on Cacao, Sixth Meeting. Bahia, Brazil, 1956*: 73-78.

Desrosiers, R. (1958) Ultimos avances en el control de enfermedades del cacao en el campo. *Report of the Inter-American Technical Committee on Cacao. Seventh Meeting. Palmira. Colombia*: 99-103.

Desrosiers, R. (1960) Fungus diseases of cacao and their control. In: Hardy, F. (Editor) *Cacao Manual*: 231-259. Turrialba, Costa Rica; Inter-American Institute of Agricultural Sciences.

Desrosiers, R.; Von Buchwald, A.; Bolaños, C. (1955) Effects of rainfall on the incidence of Monilia pod rot of cacao in Ecuador. *FAO Plant Protection Bulletin* **3**: 161-164.

Desrosiers, R.; Diaz, J. (1956) Efecto de diversos fungicidas en el control de la Monilia. *Turrialba* **6**: 19-29.

Desrosiers, R.; Diaz, J. (1957) The world distribution of diseases of cacao. *Report of the Inter-American Technical Committee on Cacao, Sixth Meeting, Bahia, Brazil, 1956*: 331-341.

Desrosiers, R.; Suárez, C. (1974) Monilia pod rot of cacao. In: Gregory, P. H. (Editor) *Phytophthora disease of cocoa*: 273-277. London, UK: Longman.

Diaz, J. (1957) Observaciones sobre le incidencia de Monilia del cacao en Ecuador. *Turrialba* **7**: 95-99.

Enriquez, G. A.; Suárez, C. (1978) Monilia disease of cocoa in Costa Rica. *Turrialba* **28**: 339-340.

Ernelholm, I. (1948) *Cacao production of South America*. Gothenburg, Sweden: Holmquists Boktryckeri.

Evans, H. C. (1978) Witches' broom disease of cocoa (*Crinipellis perniciosa*) in Ecuador. I. The fungus. *Annals of Applied Biology* **89**: 185-192.

Evans, H. C. (1980) Pleomorphism in *Crinipellis perniciosa*, causal agent of witches' broom disease of cocoa. *Transactions of the British Mycological Society* **74** (in press).

Evans, H. C.; Edwards, D. F.; Rodriguez, M. (1977) Research on cocoa diseases in Ecuador: past and present. *PANS* **23**: 68-80.

Evans, H. C.; Stalpers, J. A.; Samson, R. A.; Benny, G. L. (1978) On the taxonomy of *Monilia roreri*, an important pathogen of *Theobroma cacao* in South America. *Canadian Journal of Botany* **56**: 2528-2532.

Fowler, R. L.; Desrosiers, R.; Hopp, H. (1956) Evaluation of certain factors affecting the yield of cacao in Ecuador. *Ecology* **37**: 75-81.

Franco, T. (1958) Transmisión de la Moniliasis del cacao por el *Mecistorhinus tripterus* F. *Report of the Inter American Technical Committee on Cacao, Seventh Meeting, Palmira, Colombia*: 130-136.

García, C. (1954) Resultados de las investigaciones sobre represión de enfermedades del fruto del cacao. *Report of the Inter-American Technical Committee on Cacao, Fifth Meeting, Turrialba, Costa Rica, Document* No. 44, 6 pp.

Holliday, P. (1953) The cultivated cacao of Colombia. *Journal of the Agricultural Society of Trinidad & Tobago* **53**: 397-406.

Holliday, P. (1955) A test for resistance to *Marasmius perniciosus* Stahel. *Report of Cacao Research 1954*: 50-55. St. Augustine, Trinidad; Imperial College of Tropical Agriculture.

Holliday, P. (1970) *Monilia roreri*. *CMI Descriptions of Pathogenic Fungi and Bacteria* No. 226.

Holliday, P. (1971) Some tropical plant pathogenic fungi of limited distribution. *Review of Plant Pathology* **50**: 337-348.

Jorgensen, H. (1970) Monilia pod rot of cacao in Ecuador. *Turrialba* **15**: 4-13.

Lass, R. A. (1970) Cocoa in Ecuador. *Cocoa Growers' Bulletin* **14**: 8-15.

López, R. (1954) Fisiología de la germinación de esporas de *Monilia* sp. *Cacao en Colombia* **3**: 183-207.

Malaguti, G.; Díaz, H. (1958) Observaciones sobre las enfermedades del cacao en Venezuela. *Report of the Inter-American Technical Committee on Cacao. Seventh Meeting, Palmira, Colombia*: 163-164.

McLaughlin, J. H. (1950) Observations on cacao in Peru. *Cacao Information Bulletin* **2**: 3-4.

Meza Sanoja, C. (1973) Efecto de Benlate y Cupravit sobre el combate de la Moniliasis y Mancha de agua del cacao. *Revista de la Facultad de Agronomía, Universidad del Zulia, Venezuela* **2**: 87-94.

Moore, R. T.; McAlear, J. H. (1962) Fine structure of mycota. 7. Observations on septa of ascomycetes and basidiomycetes. *American Journal of Botany* **49**: 86-94.

Naundorf, G. (1954) Contribuciones al problema de la Moniliasis en cacao. *Cacao en Colombia* **3**:35-61.

Naundorf, G. (1955) Contribución al problema de la Moniliasis en cacao y su represión. *Cacao en Colombia* **4**: 11-14.

Orellana, R. G. (1954) Cacao disease in Venezuela, Colombia, Ecuador and Trinidad. *FAO Plant Protection Bulletin* **2**: 49-52.

Orellana, R. G. (1956) Occurrence of Monilia pod rot and other cacao diseases in eastern Panama. *FAO Plant Protection Bulletin* **4**: 168-169.

Palma, M. (1949) Report on Venezuela. *Report of the Inter American Technical Committee on Cacao, Second Meeting, Turrialba, Costa Rica*: 20-21.

Pound, F. J. (1938) Cacao and witchbroom disease (*Marasmius perniciosus*) of South America. With notes on other species of *Theobroma*. *Report on a visit to Ecuador, the Amazon valley and Colombia, April 1937-April 1938*. Port of Spain, Trinidad; Yuille's Printerie.

Preuss, P. (1901) *Expedition nach central und Sudamerika 1899/1900*. Berlin, Germany; Verlag des Kolonial-Wirtschaftlichen Komitees.

Purseglove, J. W. (1974) *Tropical crops: Dicotyledons*. 719 pp. London, UK; Longman.

Reyes, L. C. (1975) Situación fitosanitária del cultivo del cacao en Venezuela. *International Round Table on Diseases of Cocoa, Itabuna, Brazil, June 1975* (mimeograph).

Ridgway, R. (1912) *Color standards and color nomenclature*. Washington, DC, USA; Ridgway.

Rorer, J. B. (1918) Enfermedades y plagas del cacao en el Ecuador y métodos modernos apropriados al cultivo del cacao. *Report presented to the Agricultural Society of Ecuador*. Guayaquil (mimeograph).

Rorer, J. B. (1926) Ecuador cacao. *Tropical Agriculture, Trinidad* **3**: 46-47, 68-69.

Sepulveda, R. (1955) Biología del *Mecistorhinus tripterus* F. (Hem., Pentatomidae) y su posible influencia en la transmisión de la Moniliasis del cacao. *Cacao en Colombia* **4**: 15-42.

Singer, R. (1976) Marasmieae (Basidiomyceres-Tricholomataceae). *Flora Neotropica Monograph* No. 17. New York, USA; New York Botanical Garden.

Sotomayor, F. (1965) Estudios preliminares sobre la resistencia de algunos clones de cacao a la Moniliasis provocada por inoculación artificial. Ing. Agr. Thesis, University of Guayaquil, Ecuador.

Sotomayor, I. (1973) Efecto de fungicidas sistémicos en el control de la Moniliasis del cacao. Ing. Agr. Thesis, University of Guayaquil, Ecuador.
Suárez, C. (1971) Estudio del mecanismo de penetración y del proceso de infección de *Monilia roreri* Cif. & Par. en frutos de cacao *(Theobroma cacao* L.). Ing. Agr. Thesis, University of Guayaquil, Ecuador.
Suárez, C. (1972) Mecanismo de penetración y proceso de infección de *Monilia roreri* Ciferri & Parodi en frutos de cacao (*Theobroma cacao* L.). *Report, Fourth International Cocoa Research Conference, Trinidad and Tobago, January 1972;* 506-510.
Thorold, C. A. (1975) *Diseases of cocoa.* 423 pp. Oxford, UK; Clarendon Press.
Thurston, H. D. (1973) Threatening plant diseases. *Annual Review of Phytopathology* **11**: 27-52.
Van Hall, C. J. J. (1914) *Cocoa.* 361 pp. London, UK; Macmillan.
Wellman, F. L. (1972) *Tropical American Plant Diseases.* 989 pp. Metuchen, New Jersey, USA; Scarecrow Press.
Wood, G. A. R. (1959) *Cocoa-growing in Venezuela, Colombia and Ecuador, with notes on three cocoa diseases.* Bournville, Birmingham, UK; Cadbury Brothers, Ltd.
Wray, G. (1954) Algunos aspectos ecológicos del cultivo del cacao en el Ecuador. *Report of the Inter-American Technical Committee on Cacao, Fifth Meeting, Turrialba, Costa Rica, Document* No. 40, 18 pp.