Witches' brooms and frosty pods: threats to world cacao production

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On several occasions, witches' broom disease (WBD) has wrought havoc in the cacao industry of the New World. Frosty pod disease (FPD) is an equally devastating pathogen of cacao. It is not just our supply of chocolate that is at stake – people are losing their livelihoods.

Cacao (*Theobroma cacao* L. [theo-broma = food of the gods]) is a major cash crop in the moist tropics, grown mainly in West Africa, South America and South East Asia. With growing demand for chocolate and cocoa-containing products, cacao production has risen steadily. West Africa (Côte d'Ivoire, Ghana, Nigeria, and Cameroon) continues to be the main region for world cacao production with almost 70% of world supply. Indonesia is the main Asian producer having grown in production very rapidly in the 1980s and 1990s, masking a decline in Brazilian production since WBD took hold there.

New plantings and rejuvenation of old farms are taking place in the established cacao-producing countries, and new countries like Vietnam are starting to plant more cacao. From a sustainability angle, cacao is a very desirable crop for areas of high rainfall where the soil cannot tolerate short cycle crops. A recent conference at the Smithsonian Institute in Panama also highlighted the importance of cacao and other tree crops for migrant birds. The US Government has been promoting cacao in the sub-Andean region as an alternative cash crop to coca. However, in the Americas, past low prices and the susceptibility of cacao to various pest and diseases make it difficult to persuade farmers to take up or persist with cacao cultivation. Of particular concern are two fungal diseases, witches' broom disease (WBD) and frosty pod disease (FPD), which alone and in combination can cause near-total crop failure.

The cultivation and consumption of cacao was widespread in South and Central America in pre-Columbian times (including Amazonian cultures such as the Yanomami), with the beans even used as currency by the Aztecs. The Spanish adapted the original *chocolatl* drink by addition of sugar and spices, and its bitter taste and stimulant effect (due to alkaloid theobromine) became popular throughout Europe during the 16th and 17th centuries. After Cortes' conquest of the Aztecs, cacao plantations had spread to the Spanish colonies in the Caribbean (Jamaica), the Far East (Philippines) and South America (Ecuador). Only in the 19th century was cacao cultivation introduced to West Africa, now the source of most of the world's cacao. Driven by reduced levies and Van Houten's development of a method for preparing cocoa powder in 1828 (allowing more palatable drinks to be made), consumption grew during the 19th century. Ecuador emerged as the world's largest cacao growing area, producing between 20 and 50% of world cacao throughout the 19th century (20 000 tonnes yr^{-1} in 1900, world production of cacao was >2 500 000 tonnes in 2001). Great estates grew up in the coastal region of Ecuador, and the distinctive Arriba flavour of its *Cacao Nacional* still commands a premium price on world markets.

Title image. The small pink mushrooms formed by Crinipellis perniciosa, *the WBD pathogen.*

Witches' broom disease

The boom times for the Ecuadorian cacao barons came to an abrupt end when a previously unknown disease, first identified in Surinam (then Dutch Guyana) by the German pathologist Gerald Stahel, reached Ecuador in 1921. In less than a decade, Ecuadorian production was halved. Called *krulloten* disease by Stahel and *escoba de bruxa* in Spanish, the disease causes the growing parts of the cacao trees to become swollen and branched, giving the appearance of a witches' broom (hence WBD). These brooms are vivid green when young (Figure 1a) but die within one or two months (Figure 1b), imparting a brown colour to the canopy of heavily infected trees (Figure 1c). More serious is the infection of flowers and young fruits on the trunks (cacao is a cauliflorous plant), giving rise to cushion brooms (Figure 1d) or deformed pods (Figure 1e). Infection later during pod growth can lead to cryptic infection, only apparent when the pod is cut open (Figure 1f). During the wet season following infection and broom death, the pathogen reveals itself in the form of small pink mushrooms (title image), now classified as *Crinipellis perniciosa*, whose abundant basidiospores, although very intolerant of dry conditions, are highly effective propagules for the dissemination of the disease within the humid canopies of cacao plantations.

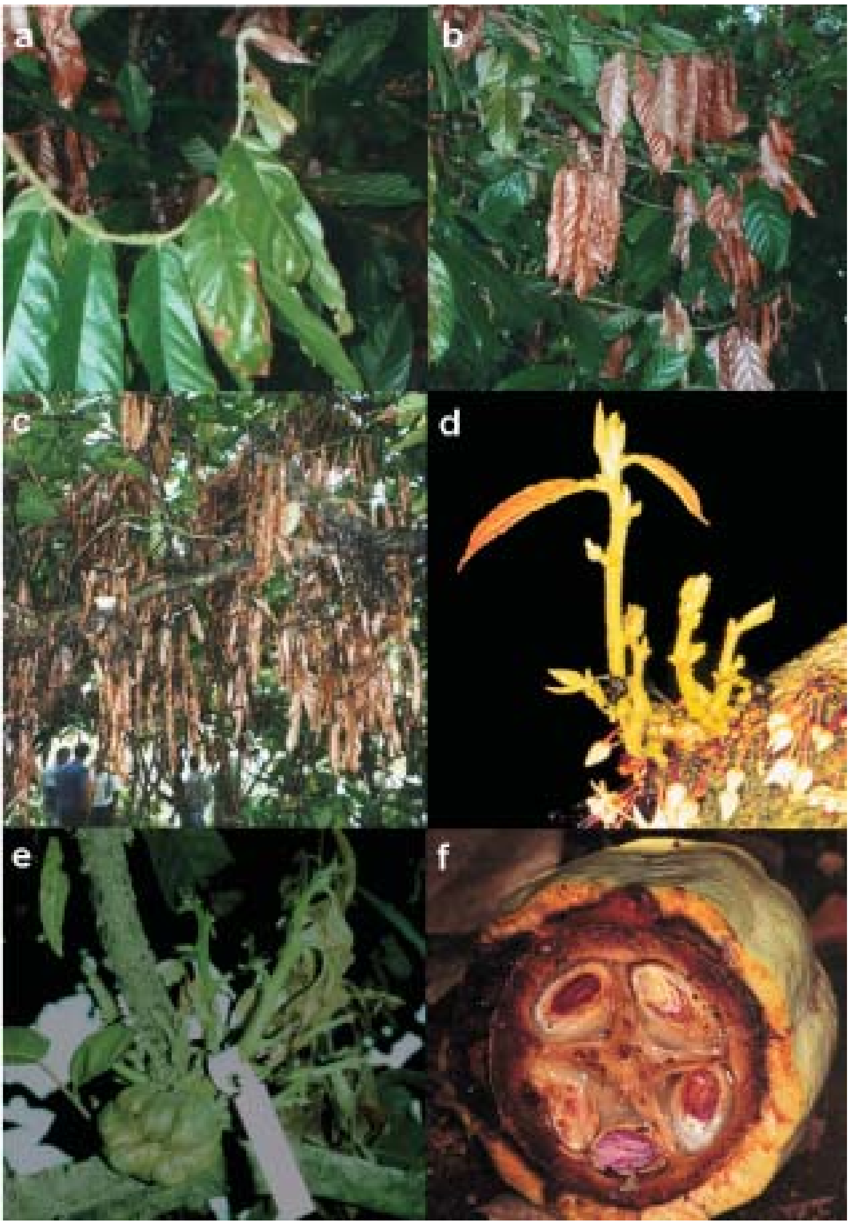

Figure 1. Symptoms of witches' broom disease. a) A green terminal broom on an infected shoot meristem. b) Attached dead brooms. c) The majority of shoots are killed under heavy infection pressure. d) An infected flower cushion, showing the development of cushion brooms. e) A developing pod which has become deformed due to infection by C. perniciosa. *f) A mature pod, which is superficially healthy but revealed to be infected when cut open.*

Agaric fungi (forming mushrooms) are relatively rare as pathogens. *C. perniciosa* is very unusual in that it attacks the green tissues of healthy host plants. Though a wide range of agaric fungi can form biotrophic infections of root tissues (*i.e.*, ectomycorrhizas) and others may latently colonise living trees, there are, to our knowledge, no clear examples of a similar behaviour in association with aerial plant tissues.

Basidiospores of *C. perniciosa* are produced in vast numbers (>10^7 per fruit body) but are not hardy propagules. Whilst they are very effective in dispersal over short distances (<1 km), it is estimated that their requirement for high humidity to retain viability and their susceptibility to solar UV-B radiation make it very unlikely that they could mediate dispersal over more than 60 km. In fact, man has been the most important vector of the disease, most likely in the form of superficially healthy but infected cacao pods (Figure 1f). The rapid spread of the disease from Surinam in 1895 to Ecuador (1921) and Trinidad (1928), attests to this. In the 1970s, coincident with the extensive deforestation and oil exploration of Amazonian Ecuador, the disease spread across the Andes, probably via migrating farmers. Similar developments in Brazil led to the establishment of cacao farms in the Amazonian states of Rondonia and Acre and the inevitable occurrence of the disease. Disastrously, expertise in cacao cultivation for these new farms was imported from Bahia on the Atlantic coast and, ultimately, the disease spread to Bahia (Figure 2).

In Bahia, where the first incidence of the disease was observed in 1989 (Pereira *et al*., 1996), the ravages of WBD have been worse than in any of the other infected regions, due in part to the high density of cacao farms in the Mata Atlântica forest region but also the absence of a distinct dry season (allowing year-round infection by the pathogen). Trees can be killed by WBD due to the continuous infection pressure, something that is hardly ever seen elsewhere. A large area centred upon the town of Ilheus (a town built on the wealth of cacao farms), has suffered economic devastation. It is estimated that 200 000 people were put out of work as a result of WBD with a further two million people being indirectly affected. Knock-on effects

Figure 2. A map of South America showing the range of WBD (dashed) and FPD (dotted). The asterisk indicated the location of Ilheus in Bahia Province. Also shown is Minas Gerais (MG) where the S-biotype of C. perniciosa *has been found.*

have included a soaring crime rate and extensive rural depopulation.

In the world of global agriculture, one man's meat is another man's poison. The tribulations of the cacao growers of South America have provided an opportunity for expansion of plantations in West Africa and Malaysia/ Papua New Guinea, though cultivation of cacao in these areas is not problem free, with insect pests (mirids and cocoa pod borers) and diseases such as black pod (*Phytophthora palmivora* and *P. megakarya*) in West Africa or vascular streak dieback (*Oncobasidium theobromae*) in the Far East causing concern. However, the great fear is that improved travel links (especially direct air travel between tropical countries) could lead to the spread of WBD to these areas. Some countries, such as Côte d'Ivoire (where cacao generates 60% of GDP), are heavily dependent on their cacao industries and the arrival of WBD would represent not only an economic but also a humanitarian disaster.

The biology of *C. perniciosa*

C. perniciosa is a hemibiotrophic pathogen, whose basidiospores are able to infect meristematic tissues of *T. cacao* and their relatives in the genera *Theobroma* and *Herrania* (all members of the family Malvaceae). The distinctive symptoms of WBD result from abnormal swelling and cell division in infected tissues, as well as loss of apical dominance. This ultimately leads to the death of the 'broom'. The symptoms are indicative of an imbalance in plant growth regulators but the mechanism *in planta* remains unknown.

The mycelium of the fungus in green brooms is composed of wide, convoluted hyphae (filaments), which are present at very low density, and grow only between host cells (Figure 3a). However, in dead brooms, narrower hyphae bearing the clamp connections characteristic of basidiomycete mycelia are present (Figure 3b) and it is these that penetrate the dead host cells, degrading them by means of cellulose and lignin-degrading enzymes. In the field, basidiomata (fruit bodies) of *C. perniciosa* are formed only during the wet season.

C. perniciosa has been found in association with several plant hosts that are quite unrelated to cacao, including the Solanaceae, Bignoniaceae, Bixaceae and Malpighiaceae. Brooms have been found on a variety of hosts throughout Brazil (including Minas Gerais, a semi-tropical area more than 1000 km south of Amazonia) (Barretto and Evans, 1996). However, spores from basidiomata produced on these brooms do not infect cacao (the reverse is also the case) but do induce brooms on most of the solanaceous hosts hitherto tested (tomato, potato, pepper, aubergine).

C. perniciosa basidiomata have also been found on the bark of woody lianas, though never in association with broom symptoms. The fungus is consistently associated with the liana *Arrabidaea verrucosa* (Bignoniaceae) and it is likely that it forms a cryptic infection of its host (Griffith and Hedger, 1994b). The fungus is also able to spread short distances from these vines onto plant litter falling from the rainforest canopy by means of crimson-pigmented mycelial pads. This unusual colonisation strategy allows the fungus to spread to and degrade new resources. It has been noted that *C. perniciosa* and other basidiomycetes (*e.g.*, members of the genus *Marasmius*) that inhabit the rainforest understorey can form aerial 'nets', which trap substantial amounts of falling litter.

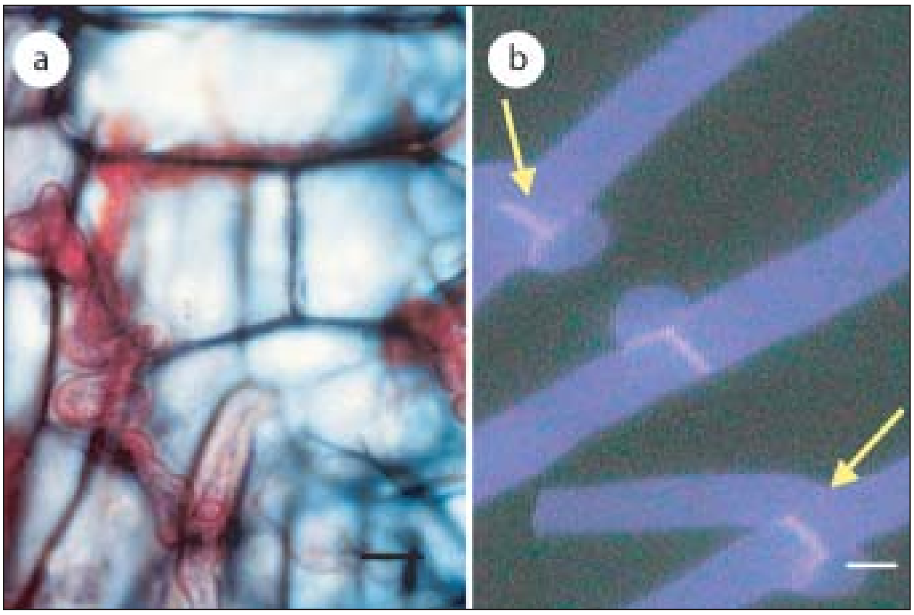

Figure 3. (a) The intercellular mycelium of C. perniciosa *(stained with Congo Red) proliferating within a green cacao broom. Scale bar = 10 μm. (b) The narrower saprotrophic mycelium of* C. perniciosa *(stained with Calcofluor and viewed under UV epifluoresence microscopy) with clamp connections (arrowed). Scale bar = 2 μm.*

Several lines of evidence indicate that *C. perniciosa* consists of several distinct host-specific biotypes (Griffith and Hedger, 1994a). Unlike the C- (cacao) and S- (*Solanum*) biotypes, which exhibit a non-outcrossing (selfing) breeding strategy, the L- (liana) biotype, in common with the majority of basidiomycete fungi, is outcrossing. Thus a colony emanating from a single L-biotype basidiospore must mate with a compatible mycelium before its life cycle can be completed. In contrast, colonies originating from single basidiospores of the non-outcrossing C- and S-biotypes can form basidiomata without mating. This is of crucial importance in the context of WBD epidemiology, since all infected cacao meristems can give rise to basidiomata and thus disseminate the pathogen.

Examination of izoenzyme profiles and somatic incompatibility also suggest that the three biotypes are distinct, and that the non-outcrossing C- and S-biotypes are less diverse than the outcrossing L-biotype. These suspicions have been confirmed by our phylogenetic analyses using DNA sequence data from the ribosomal RNA (rRNA) locus. C-biotype samples taken throughout tropical South America were nearly all identical (Figure 4), whereas L-biotype isolates from a single site were clearly distinct. This indicates that the C- and S-biotypes may have evolved relatively recently, possibly from a rainforest-dwelling ancestor like the L-biotype. It is hoped that future fieldwork will identify this ancestor.

Disease control

Once WBD becomes established in a plantation, crop yield can decrease by up to 90% and, despite a century of research, no truly effective control strategy has been devised. Fungicide application is rather impractical for tropical tree crops and ineffective in areas of high rainfall. Control of the disease by pruning of brooms has been found to be partially effective, though costly in labour. The ideal solution to the problem of WBD would be cacao varieties which are high-yielding yet immune to the pathogen.

Extensive programmes to collect and screen 'wild' cacao varieties from Western Amazonia have identified resistant cultivars but, as with all tree crops, breeding programmes are slow and it is difficult to combine resistance traits with those of good bean flavour and high yield. In Ecuador, one resistant variety CCN51 is extensively cultivated by grafting onto susceptible rootstock but it lacks the prized Arriba flavour. Similarly, in Brazil, several clones with elevated resistance have been developed in recent years. With recent advances in somatic embryogenesis and micropropagation techniques, it is possible to multiply up and deploy these resistant clones much more rapidly than was previously possible. The fact that strains of the C-biotype of *C. perniciosa* show little genetic diversity, combined with the pathogen's long life cycle (ca. 12 months), does hold some hope that once identified and disseminated, any resistant genotypes would prove stable in the field.

Biological control offers a fresh approach. *Trichoderma stromaticum* is an effective hyperparasite of the mycelium and basidiomata of *C. perniciosa* (Sanogo *et al.*, 2002). Trials are currently underway in Brazil to optimise the application and establishment of *T. stromaticum* on susceptible cacao tissues. A parallel USDA-funded project is exploring the use of endophytic (living in plants) fungi to protect against WBD. A range of fungi inhabit the internal tissues of cacao meristems. Although these infections are asymptomatic (rather like the L-biotype described above), it is found that the presence of endophytes in meristematic tissues can inhibit establishment and spread of *C. perniciosa*. It is hoped that cacao seedlings inoculated with endophytic fungi will confer long-term protection against WBD.

Frosty pod disease

Frosty pod disease (FPD), caused by *Moniliophthora roreri*, is more westerly in its distribution (Ecuador, Colombia, Peru, Costa Rica) than WBD and attacks only developing pods. The disease manifests itself in the form of brown spreading lesions on the pod surface and ultimately the production of cream-coloured powdery spores (Figure 5). Sometimes swellings appear on the pod surface, prior to the formation of the asexual spores (conidia). Originally described by Ciferi and Parodi in 1933, the pathogen was named *Monilia roreri*. However, a detailed study of hyphal ultrastructure and the process of conidium formation led to is classification as *Moniliophthora roreri* (Evans *et al.*, 1978).

Until recently, FPD received less attention than WBD, although it is no less damaging. In areas where the diseases co-occur, more pods are lost to FPD and control is more difficult than for WBD, because the spores are often the first sign of infection. *M. roreri* was first officially reported in coastal Ecuador in 1917 by J B Rorer, though the disease may have been present in the area more that 20 years earlier (Evans, 1981). Like WBD, FPD has spread north and south along the coastal regions of Pacific South America and is now spreading into adjacent areas of Amazonia. The disease is present in parts of Venezuela and its continued spread through Amazonian Peru suggests an imminent threat to the plantations of Eastern Brazil. Unlike WBD, FPD has crossed the Darien gap and reached the plantations of Panama, Nicaragua and Costa Rica. Like WBD, it is possible that humans have acted as unwitting vectors in the northerly spread of FPD.

Evans (1981) noted the similarities between *M. roreri* and *C. perniciosa* in that both fungi form a distinctive swollen, convoluted and intercellular mycelium during biotrophic infection of host tissues. As part of our studies of genetic diversity within *C. perniciosa*, we included *M. roreri* (with the aim of clarifying its basidiomycete affiliation) and were surprised by how closely these two cacao pathogens are related (Figure 4). *M. roreri* is probably the nearest known relative of *C. perniciosa*.

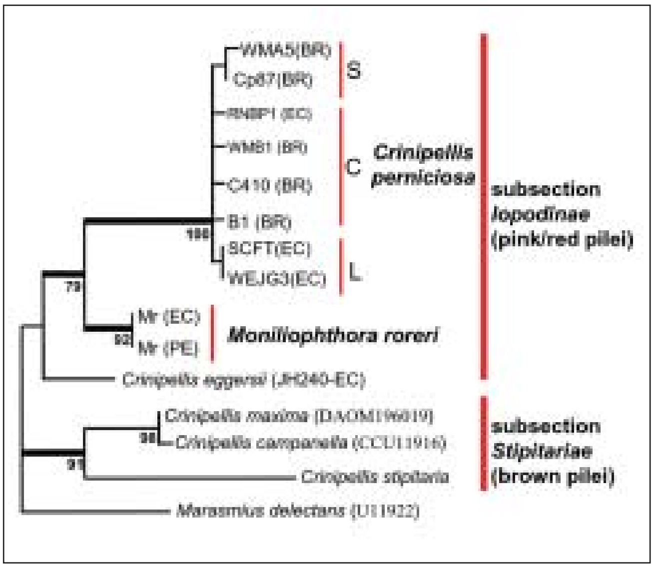

Figure 4. A maximum parsimony tree generated by phylogenetic analysis of part of the large ribosomal subunit of the rRNA locus. Percentage bootstrap (confidence) values are shown at nodes. A member of the related genus Marasmius is used as an outgroup.

Within Kingdom fungi there are many examples of 'mitosporic' fungi in which no sexual reproduction has been detected and which appear to reproduce solely by formation of conidia. For instance, *Aspergillus fumigatus*, an important human pathogen is mitosporic but DNA sequence comparisons have shown that it is related to sexually reproducing species of the genus *Neosartorya*. Thus, it is believed that the ancestor of *A. fumigatus* lost the ability to reproduce sexually. A similar explanation might seem appropriate for the relationship between *M. roreri* and *C. perniciosa* were it not for a recent discovery by Evans *et al.* (2002) that meiosis occurs within the spores of *M. roreri*, indicating that the structures previously called conidia (by definition asexual spores) are in fact the products of sexual reproduction. Thus it would appear than the ancestor of *M. roreri* lost basidioma formation but retained meiosis.

Conclusions

Cacao is traded as an international commodity and the price fluctuates according to the laws of supply and demand, as well as a host of other political and economic factors. Other cacao-producing countries have filled the gap in world cacao supply created by diseases in the Americas, so in world terms the tribulations of the South American industry is unlikely to affect supplies at a global level (though manufacturers can come to depend on an origin for a particular quality or flavour). However, the threat posed by WBD and FPD does need to be addressed in case they spread to other parts of the world. The close relatedness of the causal organisms offers some hope, since at a cellular and biochemical level both pathogens are likely to be very similar and thus susceptible to similar anti-fungal/ biocontrol agents or novel host resistance factors.

Figure 5. A developing pod infected by M. roreri *and showing symptoms of frosty pod disease. Note the cushion broom caused by infection of a flower cushion by* C. perniciosa *(Quevedo, Ecuador).*

The sequencing of the genome of *C. perniciosa* is well underway in Brazil and the improved understanding of its biology that will stem from this project will hopefully lead to the discovery of the Achilles' heel of this unusual and beautiful pathogen, as well as that of its relative *M. roreri*.

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The National Migratory Birds Centre at the Smithsonian Institute Panama – details the role of plantation cacao for migratory birds

www.lge.ibi.unicamp.br/vassoura/index.html
Website of the *Crinipellis perniciosa* genome-sequencing programme.

www.oardc.ohio-state.edu/cocoa/welcome.htm
Excellent website describing integrated pest management strategies for cacao

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