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Witches' brooms and frosty pods: two major pathogens of cacao

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Abstract The agaric *Crinipellis perniciosa* (Tricholomataceae) is a hemibiotrophic pathogen which causes witches' broom disease of cacao and has recently decimated the Brazilian cacao industry. In addition to the pathogenic cacao (C-) biotype, other biotypes are found in association with unrelated plant taxa, notably bignoniaceous lianas (L-biotype), solanaceous hosts (S-biotype), and the shrub *Heteropterys acutifolia* (H-biotype). The C- and S-biotypes are non-outcrossing and form broom symptoms on hosts, whereas the L-biotype is outcrossing and asymptomatic. Phylogenetic analysis of several regions of the rRNA locus revealed near identity between C- and S-biotype isolates from diverse locations, with the L- and H-biotypes forming separate groupings. Preliminary analysis of sequence data from *Moniliophthora roreri*, causal agent of frosty pod disease, indicates that this morphologically distinct pathogen may be closely related to *C. perniciosa*. Similarities in host infection between *C. perniciosa* and *M. roreri* have previously been noted but it is difficult to reconcile the gross morphological differences. Pairings between *C. perniciosa* and *M. roreri* gave rise to a clamped dikaryotic mycelium suggestive of a hybridisation event.

Keywords rRNA sequences; phylogenetics; endophytes; disease control; tropical ecology

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INTRODUCTION

The world's largest cocoa-producing countries are in the Northern Hemisphere (Côte d'Ivoire and Ghana). However, the focus of global production is experiencing a southward shift as production in the Far East (Malaysia, Papua New Guinea) increases, and trial plantations have been established in northern Queensland (D. Guest pers. comm.). The origins of the cacao (*Theobroma cacao* L., Malvaceae) lie in Amazonia, and until the 1920s most of the world's cocoa came from the southern neotropics, notably coastal Ecuador and Bahia Province in Brazil (Lass 1985). Both regions have suffered a significant decline in production, mainly due to the ravages of witches' broom disease (WBD) caused by the agaric fungus *Crinipellis perniciosa* (Stahel) Sing.

The German pathologist Gerald Stahel, working in Surinam (Dutch Guyana) at the start of the last century, was the first to study WBD in detail (and to name the causal agent *Marasmius perniciosus* (Stahel 1915)), though there is evidence that the disease had been recorded in Amazonia as early as 1785 (Purdy & Schmidt 1996). By 1921 the disease had reached Ecuador (then the world's largest cocoa producer) and in less than a decade Ecuadorian production was halved (Baker & Holliday 1957). Called *krulloten* disease by Stahel (1915) and *escoba de bruja* in Spanish, the fungus causes the meristematic tissue of cacao trees to become swollen (hypertrophy and hyperplasia of host cells) and branched (due to loss of apical dominance), giving the appearance of a witches' broom. These brooms

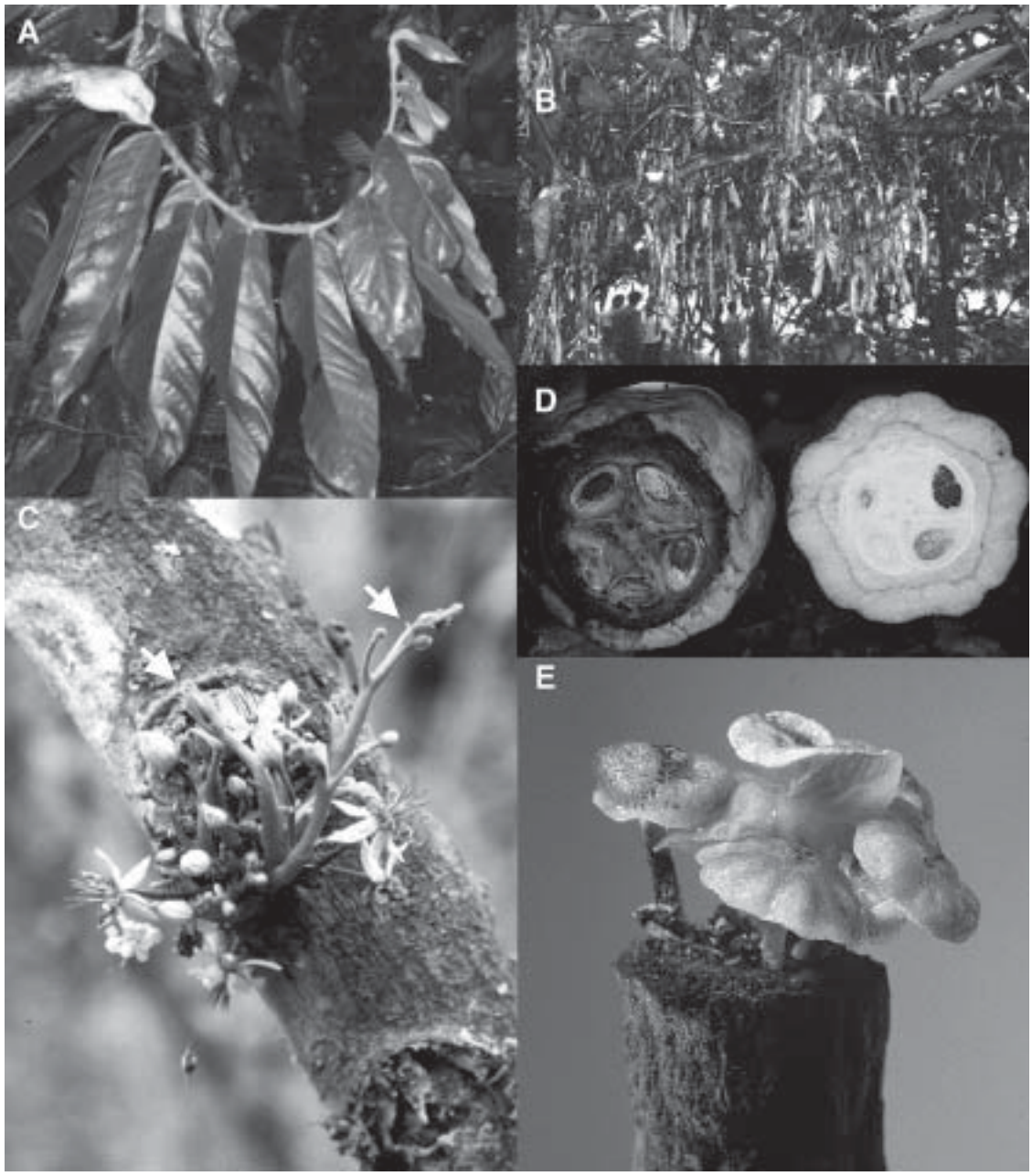

Fig. 1 Symptoms of witches' broom disease on cacao. **A**, a green terminal broom with a swollen, deformed stem; **B**, mass of dead brooms on heavily infected tree in Bahia; **C**, an infected flower cushion with some healthy flowers and others (arrowed; "cushion" brooms) are swollen and deformed; **D**, diseased pod; **E**, basidiocarps of *Crinipellis perniciosa*.

are vivid green when young (Fig. 1A) but die within 1–2 months, imparting a brown colour to the canopy of heavily infected trees (Fig. 1B). More serious is the infection of flowers and young fruits on the trunks (cacao is cauliflorous), giving rise to cushion brooms (Fig. 1C) or blackened and indurated pods. Infection of more mature pods leads to more cryptic infection visible only when pods are harvested

(Fig. 1D). During the wet season following infection and broom death, the pathogen reveals itself in the form of small pink mushrooms, re-classified as *Crinipellis perniciosa* (Tricholomataceae) by Singer (1942) (Fig. 1E).

In the world of plant pathology, most important pathogens are ascomycetes (e.g., powdery mildews), oomycetes (e.g., downy mildews), or heterobasidiomycetes (rusts, smuts). Agaric fungi (forming mushrooms) are relatively rare as pathogens, the best known in the Southern Hemisphere being the honey fungus (*Armillaria mellea* species complex), a root pathogen which attacks and kills a wide range of tree species by means of subterranean rhizomorphs (Coetzee et al. 2001). In this context *C. perniciosa* is very unusual in that it attacks and deforms the green tissues of healthy host plants. Though a wide range of agaric fungi can form biotrophic infections of root tissues as ectomycorrhizas (Smith & Read 1997), there are to our knowledge no clear examples of similar behaviour in association with aerial plant tissues. However, several lines of evidence suggest that cryptic associations between basidiomycetes and woody plants are far more widespread than was previously thought (Boddy & Rayner 1983; Petrini 1986; Zhang et al. 1997).

Basidospores of *C. perniciosa* are not hardy propagules, but over short distances within the humid canopies of cacao plantations, they disseminate the disease most effectively. However, their requirement for high humidity (>99% RH) 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 (Frias et al. 1991; Andebrhan et al. 1993). In fact, people have been the most important vector of the disease, most likely in the form of asymptomatically infected cacao pods. The rapid spread of WBD from Surinam in 1895 to Ecuador (1921) and Trinidad (1928) attests to this (Baker & Holliday 1957). In the 1970s, coincident with the extensive deforestation and oil exploration of Amazonian Ecuador, the disease spread across the Andes, probably via migrating farmers (Griffith et al. 1994a). Similar developments in Brazil led to the establishment of cacao plantations in the Amazonian states of Rondonia and Acre and the inevitable occurrence of the disease (Rudgard 1986). Disastrously, expertise in cacao cultivation was imported from Bahia on the Atlantic coast for these new plantations and ultimately the disease spread to Bahia, very probably in the form of diseased, deformed pods carried by labourers returning to visit their families.

In the coastal Mata Atlântica forest zone of Bahia, where the first incidence there of the disease was observed in 1989, the high density of cacao plantations and the absence of a distinct dry season have conspired to make the ravages of WBD worse than in any of the other infected regions (Pereira et al. 1990). Trees can be killed by WBD due to continuous infection pressure, which is rarely seen elsewhere. A large area centred upon the town of Ilheus has suffered economic devastation due to WBD (Pereira et al. 1996), where it is estimated that 200,000 people were put out of work. Knock-on effects have included a soaring crime rate and extensive rural depopulation (Brazilian Ministry of Agriculture 2002). In little over a decade Brazil has become a net importer of cacao, having been the world's second largest producer as recently as the early 1990s (Wakeling 1994). It is reasonable to claim that WBD is the most important plant pathogen to have afflicted the Southern Hemisphere in recent decades. Whilst illustrating the potential threat to cacao plantations in Asia and Africa, a risk which is exacerbated by increased inter-continental trade and air travel, the recent disaster in Bahia has breathed new life into research on this fungus.

AETIOLOGY OF THE DISEASE

Crinipellis perniciosa is a hemibiotrophic pathogen whose basidiospores are able to infect meristematic tissues of *Theobroma cacao* and various species in the genera *Theobroma* and *Herrania* (all members of the family Sterculiaceae). The distinctive symptoms are the result of hypertrophy and hyperplasia of infected tissues, and loss of apical dominance, ultimately leading to death of the "broom" and its subsequent saprotrophic exploitation by the pathogen (Wheeler 1985). In living host tissues the density of fungal mycelium is very low and it is restricted to cortical regions where it grows intercellularly (Calle et al. 1982; Penman et al. 2000).

Though the symptoms are suggestive of an imbalance in plant growth regulators (e.g., destruction of auxins or release of cytokinins by the fungus), the mechanism of symptom production *in planta* remains unknown (Orchard et al. 1994). Also unclear is whether broom death is the result of production of fungal toxins or accelerated host senescence (Evans 1980). We have established that "pathogenesis related" (PR) proteins are present in green brooms, suggesting that the host is attempting to mount a defence response (R. Birch unpubl. data).

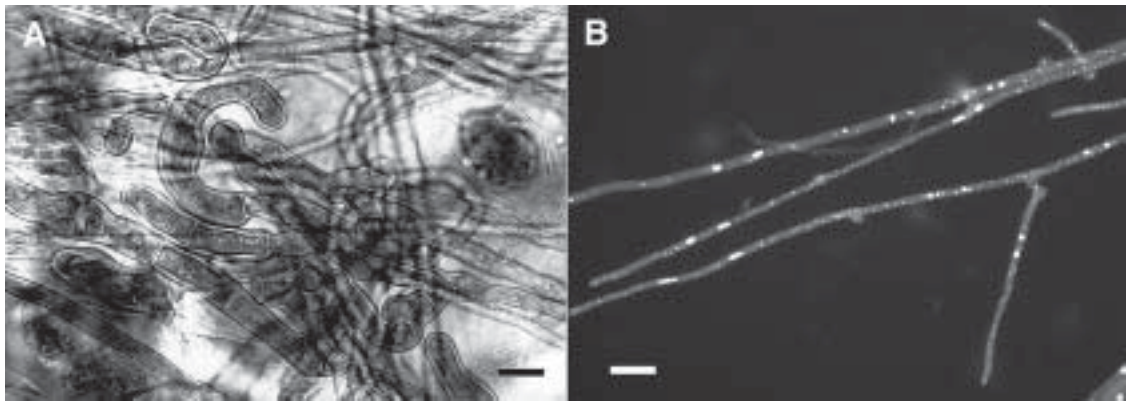

Fig. 2 **A**, Swollen, convoluted hyphae found within green brooms; **B**, narrower, clamped, dikaryotic hyphae found in dead broom tissues (dual stained with Calcofluor White M2R and DAPI). Scale bar = 10 μm.

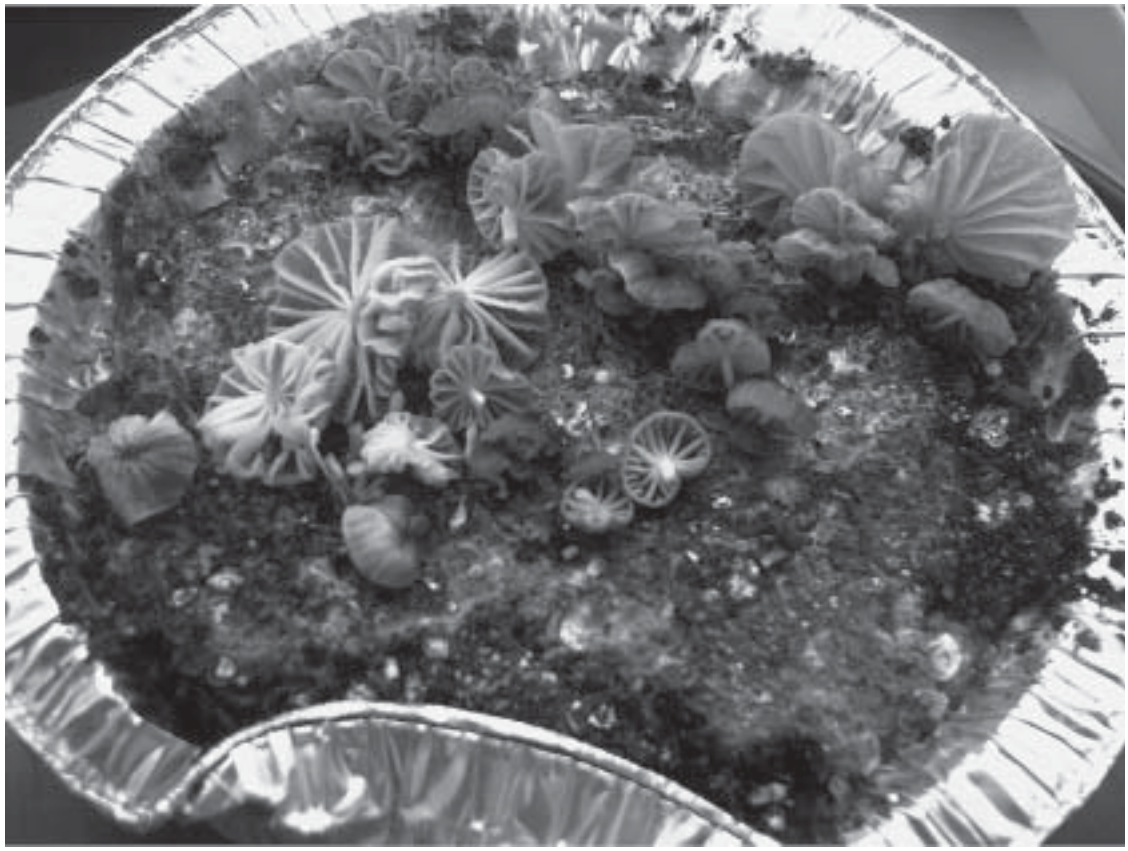

Fig. 3 Basidiocarps of *Crinipellis perniciosa* formed on "piedish" cultures following cycles of wetting/drying to simulate tropical conditions.

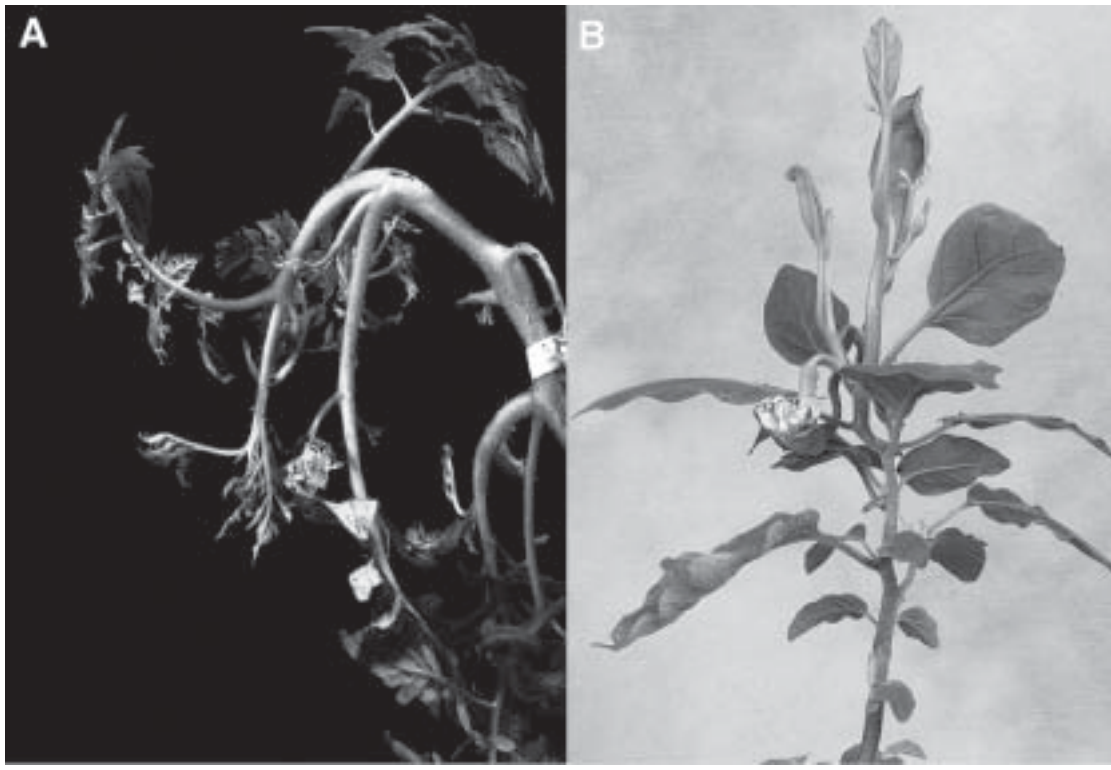

Fig. 4 Symptoms of the S-biotype of Crinipellis perniciosa on solanaceous hosts. **A**, tomato; **B**, aubergine.

The mycelium of the fungus in green brooms is rather distinctive in appearance with wide, convoluted hyphae (Fig. 2A) (Griffith & Hedger 1994a). However, in dead brooms narrower hyphae bearing the clamp connections characteristic of basidiomycete mycelia are present (Fig. 2B) and it is these that colonise and degrade the host tissues by white rotting (Bravo & Hedger 1988).

In the field, basidiocarps of *C. perniciosa* are formed only during the wet season. Fruiting of the fungus under laboratory conditions relies upon the use of intermittent wetting and drying (on a bran-based medium; Fig. 3) which simulate the large diurnal variations in broom moisture content that occur in the canopy of a cacao tree (Rocha & Wheeler 1985; Griffith & Hedger 1993). The ability of the mycelium to tolerate conditions of low and fluctuating water availability (including freezing) (Bravo & Hedger 1988) is shared with a number of other tropical basidiomycetes (e.g., several members of the related genus *Marasmius*) which inhabit plant litter suspended above the ground in the rainforest understorey (Hedger et al. 1994).

HOST RANGE AND BIOTYPES

Crinipellis perniciosa has been found in association with several plant hosts in families which are quite unrelated to cacao (Sterculiaceae), including Solanaceae (Bastos & Evans 1985), Bignoniaceae (Griffith & Hedger 1994b), Bixaceae (Bastos & Andebrhan 1986), and Malpighiaceae (Resende et al. 2000). Brooms similar to those on cacao have been found on a variety of woody and herbaceous hosts belonging to the Solanaceae (many hosts) and Malpighiaceae (*Heteropterys acutifolia*) throughout Brazil, including semi-tropical Minas Gerais province, which lies more than 1000 km south of Amazonia. In the case of solanaceous hosts, brooms are formed on a wide range of hosts including tomato, potato, pepper, and aubergine, though the morphology of the broom is dependent on the growth patterns of the host (Fig. 4A,B).

Griffith & Hedger (1994c) initiated the use of acronyms to describe the various biotypes of *C. perniciosa* according to their hosts, for example, C-biotype on cacao and L-biotype on lianas. Whilst it

is widely observed that inoculation of non-hosts (e.g., S-biotype spores inoculated onto cacao; (Bastos & Evans 1985)) gives rise to some hypertrophy at the site of inoculation, there are contradictory reports as to the host-specificity of some biotypes. For instance, spores from basidiocarps on brooms of the shrub *Solanum paniculatum* were found to cause broom formation on both cacao and solanaceous hosts (Silva et al. 1992). Similarly, Resende et al. (2000) found that an isolate of *C. perniciosa* obtained from *H. acutifolia* did cause broom symptoms in cacao but only on varieties highly susceptible to WBD. The single report of brooms on *Bixa orellana* (Bixaceae) (Bastos & Andebrhan 1986) is difficult to assess since a *C. perniciosa* strain isolated from one of these brooms was somatically compatible with C-biotype isolates from the same region (Griffith 1989). Nevertheless, the weight of evidence suggests that the host ranges of these two biotypes are distinct.

Crinipellis perniciosa basidiocarps have also been found on the bark of woody lianas (in primary and secondary rainforest) in several countries, though it has only once been seen causing broom symptoms (H. Evans pers. comm.). In several locations in coastal and Amazonian Ecuador, the fungus was consistently associated with vines of *Arrabidaea verrucosa* (Bignoniaceae) (Griffith & Hedger 1994b). Basidiocarps are often found on debris of various origins, which are attached to these vines by means of crimson-pigmented pseudosclerotial plates, indicating a novel mechanism of mycelial spread from the bark of living liana vines into fallen litter. In the case of the liana (L-) biotype of *C. perniciosa* and other understorey basidiomycetes, substantial amounts of falling litter may be trapped and exploited in this manner (Hedger et al. 1994). Spores from L-biotype basidiocarps do not form brooms on either cacao or tomato, though cankers are formed when cacao seedlings are infected (Evans 1977). However, in common with C- and S- biotype basidiospores, they are able to form a prolonged biotrophic infection of potato callus, forming distinctive convoluted and swollen intercellular growth similar to that found in green brooms (Griffith & Hedger 1994a).

Detailed examination of C-, S-, and L-biotype basidiocarps has not revealed any significant morphological differences, except the slightly larger size and darker hue of the L-biotype (Hedger et al. 1987). However, several lines of evidence suggest that *C. perniciosa* is a species complex, consisting of several distinct host-specific biotypes. The broom-forming biotypes (C-, S-biotypes, as well as the less well understood B- (*Bixa*) and H- (*Heteropterys*) biotypes) exhibit a non-outcrossing breeding strategy, whereas the L- (liana) biotype, in common with the majority of basidiomycete fungi, is outcrossing (Griffith & Hedger 1994c). 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 biotypes can form basidiocarps without mating. This is of crucial importance in terms of the epidemiology of WBD, as all infected cacao meristems can potentially give rise to basidocarps and, thus, disseminate the pathogen, whereas an outcrossing fungus would only produce basidiocarps after having been infected by at least two basidiospores.

Several other lines of evidence also suggest that the various biotypes are distinct, with the broom-forming biotypes being more closely related to each other than to the non-pathogenic L-biotype. Griffith et al. (1994a) hypothesised, based on evidence from isoenzyme polymorphisms (Griffith 1989), somatic compatibility pairings (McGeary & Wheeler 1988; Griffith 1989), and mitochondrial DNA analyses (Griffith et al. 1994b), that the level of genetic variation present within and between strains of the C- and S-biotypes was very low. Our more recent phylogenetic analyses of sequence data from the ribosomal RNA (rRNA) locus (J. Nicholson unpubl. data) support this hypothesis, as do the sequence data published recently by de Arruda et al. (2003a) based on the intergenic spacer region (IGS) of the rRNA locus. de Arruda et al. (2003a) have also shown that C- and S-biotype isolates are more closely related to each other than to the H-biotype. The outcrossing and non-pathogenic L-biotype, which has been found to be show a high level of genetic variability by examination of somatic compatibility groupings and isoenzymes (Griffith 1989; Griffith & Hedger 1994b), showed similar variability at the genetic level. As can be seen from a short tract of the sequence from the ITS1 region (Fig. 5; J. Nicholson unpubl. data), sequence polymorphisms were present between isolates collected only a few metres apart in the field.

The aim of many of these studies of genetic variability within the *C. perniciosa* species complex has been to establish where the disease originated and how it has spread. Griffith et al. (1994a) have speculated, based on the low density of cacao trees in rainforest and the rarity of brooms on wild trees,

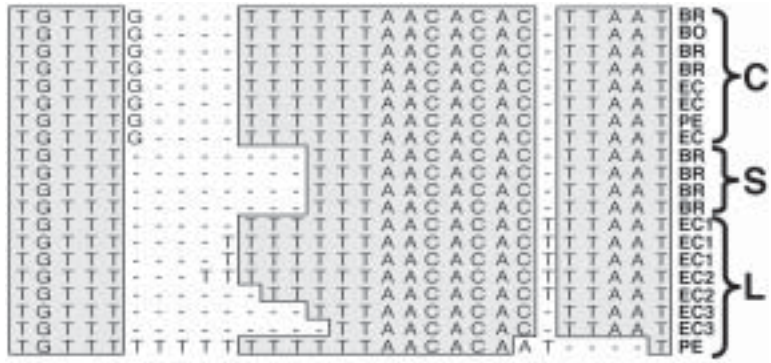

Fig 5 **A** short tract of sequence alignment from the ITS1 region of the rRNA locus of several *Crinipellis perniciosa* isolates. The C- (cacao), S- (solanaceous), and L- (liana) biotype sequences are bracketed. Labels for each sequence indicate the country of origin of each sequence as follows: BR, Brazil; BO, Bolivia; EC, Ecuador; PE, Peru. The Ecuadorian L-biotype samples are divided into groups (EC1–EC3). These sites were individual liana thickets from which multiple samples were obtained (J. Nicholson unpubl. data).

that the C-biotype may only have emerged as a result of cacao cultivation by humans. However, genetic polymorphism has been observed within C-biotype populations in Brazil using both RAPD (Andebrhan et al. 1999) and ERIC (de Arruda et al. 2003b) PCR fingerprinting, as well as AFLP analysis (J. Nicholson unpubl. data). These lines of evidence support the suspicion that WBD was transmitted to Bahia from Amazonia but at present do not provide insight as to the centre of origin of the disease at the start of the last century.

FROSTY POD DISEASE

Frosty pod disease (FPD), caused by *Moniliophthora roreri*, is more westerly in its South American distribution (Ecuador, Colombia, Peru, Costa Rica). Like *C. peniciosa*, *M. roreri* is a hemibiotroph which attacks the developing pods, though not apical meristems. The disease manifests itself as brown, spreading lesions on the pod surface, and, ultimately, produces cream-coloured powdery spores (Fig. 6A). Sometimes swellings appear on the pod surface, prior to the formation of the conidia. Originally described by Ciferri & Parodi (1933), the pathogen was named *Monilia roreri* for the formation of long chains of conidia (Fig. 6B). However, a reappraisal by Evans et al. (1978), which found the presence of dolipore septa (characteristic of Homobasidiomycete fungi) and the process of conidium formation to be inconsistent with its classification in the form-genus *Monilia*, led to its reclassification as *Moniliophthora roreri*.

Until recently FPD has 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 occurrence of sporulating lesions is often the first sign of infection (Maddison et al. 1995). *M. roreri* was first officially reported in coastal Ecuador in 1917 by J. B. Rorer, although 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 also more recently into the 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 Western Brazil. Unlike WBD, the role of people as a possible vector in this northerly spread is not clear.

Evans (1981) examined the aetiology of the disease and noted the similarities between *M. roreri* and *C. perniciosa* in that both fungi form distinctive swollen, convoluted, and intercellular mycelium during biotrophic infection of host tissues. As part of our phylogenetic analyses of the rRNA genes of *C. perniciosa*, we included *M. roreri*, with the aim of clarifying its basidiomycete affiliation. We were surprised that these two cacao pathogens appear to be quite closely related and are conducting phylogenetic analyses (J. Nicholson unpubl. data) to clarify the situation. The fact that our sequences deposited in the Genbank database (accession nos. AF335590 (Cp) and AY194150 (Mr)) show >90% identity to each other suggest that *M. roreri* may even lie within the *Crinipellis* clade. By further taxon sampling it is hoped that we can clarify this situation. There are many examples of mitosporic fungi in which no sexual stage is known (e.g., the human pathogen *Aspergillus fumigatus*), where

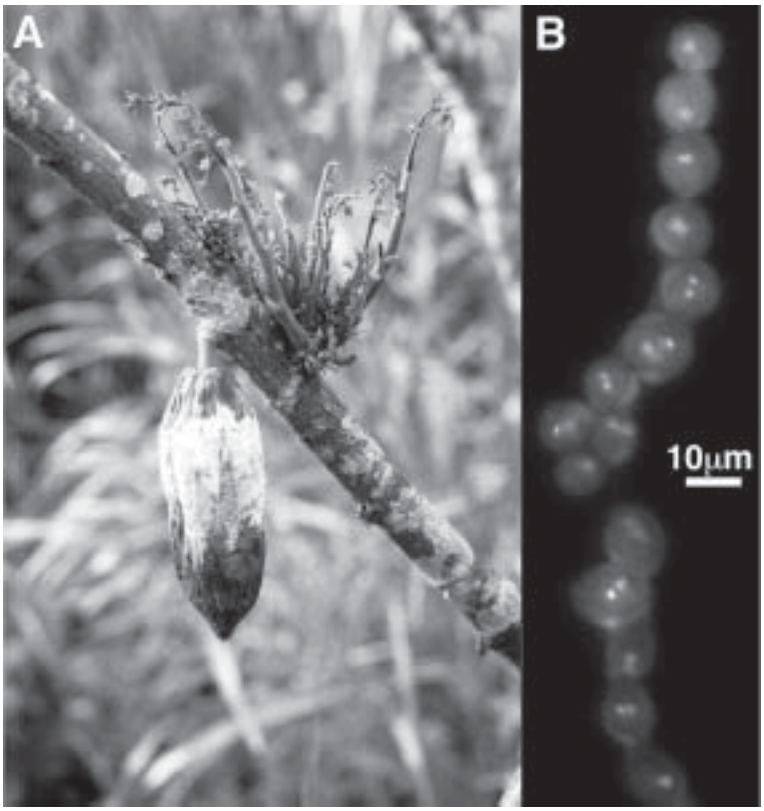

Fig. 6 **A**, Cacao pod showing a white powdery lesion cuased by *Moniliophthora roreri*. Above the pod is a cushion broom caused by *Crinipellis perniciosa* (in Quevedo Ecuador); **B**, Conidia of *Moniliophthora roreri* stained with DAPI, showing variable nuclear condition.

reproduction appears to be exclusively by formation of asexual spores (conidia). However, DNA sequence comparisons have recently revealed the evolutionary affiliations of many mitosporic fungi. Evans et al. (2002) have recently found evidence that meiosis occurs within the spores of *M. roreri*, a phenomenon which is consistent with the variable nuclear content of the spores (Fig. 6B). Therefore, it may be incorrect to refer to these structures as conidia (by definition the products of mitosis) in future. Thus, it would appear that an ancestor of *M. roreri* lost the ability to form a basidiocarp but not the ability to undergo meiotic nucelar divisions.

PUTATIVE HYBRIDISATION EVENTS

Given the potentially close taxonomic relatedness of these two fungi, we attempted crosses between isolates of *M. roreri* (whose hyphae are uninucleate and lack clamps at all times) and primary mycelia derived from single basidiospore isolates of the outcrossing L-biotype of *C. perniciosa* (which is also uninucleate and lacking clamps). After prolonged incubation (2–3 months on 3% malt agar), subculturing from interaction zones led to the outgrowth of clamped, dikaryotic mycelia, which suggested that hybridisation had occurred (G. Griffith unpubl. data). These "hybrids" remained stable during subculture for periods of several months. Morphologically, the "hybrids" were intermediate in appearance compared with the parents (Fig. 7), forming pigmented aerial mycelia (like *M. roreri*) but no conidia. However, genetic analysis of the ITS region of several of the putative hybrids matched only *M. roreri*, and there was no evidence of the presence of any DNA from the *C. perniciosa* parent. Furthermore, prolonged subculture of some hybrids led to the disappearance of the clamp connections and restoration of a typical *M. roreri* mycelium with apparently normal spores (G. Griffith unpubl. data). Rayner and colleagues (Ainsworth et al. 1990; Ramsdale & Rayner 1994) found that "wide" crosses between partially

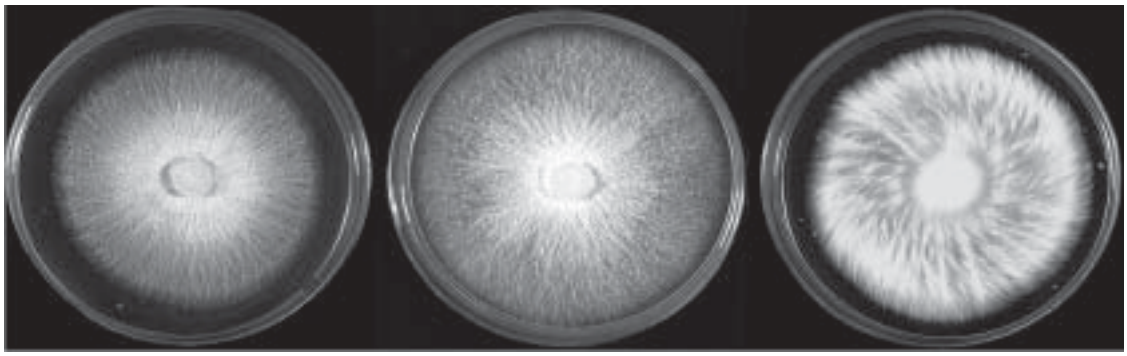

Fig. 7 Petri dish cultures showing the morphology in culture of *Moniliophthora roreri* (left), *Crinipellis perniciosa* (right), and the intermediate appearance of the putative hybrid (centre).

interfertile mycelia (in their case from the genera *Stereum* and *Heterobasidion*) gave rise to some unusual outcomes, which they attributed to "genomic conflict". It may be the case that similar processes are occurring in these *C. perniciosa*/*M. roreri* interactions.

Whilst we still have many unanswered questions about the precise nature of these putative hybridisation events, the possibility may be considered that such events could occur in nature. The geographical ranges of *M. roreri* and *C. perniciosa* (C- and L-biotype but not S-biotype) show considerable overlap. It is important to note that our experiments were conducted using the L-biotype (rather than the C-biotype) of *C. perniciosa*. The C-biotype is non-outcrossing (primary homothallic) and single basidiospore isolates form clamp connections without mating after 1–2 weeks on agar media (Griffith & Hedger 1994c). Thus, scoring of the putative hybridisation by observation of clamp connections would not be appropriate in these experiments. However, we have not observed mycelia with intermediate morphology in *M. roreri* × C-biotype crosses (G. Griffith unpubl. data). The situation *in planta* is less easy to interpret, as the biotrophic mycelium is convoluted and swollen in both species. It is, thus, difficult to establish whether the process of autodikaryotisation observed in the C-biotype on agar plates occurs in living host tissues, or whether it is simply the case that clamped hyphae are not manifest under these conditions (many basidiomycetes lack clamps even in the dikaryotic state) (Griffith & Hedger 1994c). It is very likely that mycelia of the two pathogens come into contact with each other during the infection of young cacao pods, and that hyphal anastomosis could occur. However, any resultant hybrids would probably be sterile and unable to disperse, as our efforts to induce either basidiocarp or conidium production from the putative hybrids have hitherto been unsuccessful.

DISEASE CONTROL

Once WBD is established in a plantation, crop yield generally shows a >90% decrease. Despite a century of research no truly effective control strategy has been devised (Lass 1985). Fungicide application is rather impractical for tropical tree crops and ineffective in areas of high rainfall, though protection of developing pods by fungicide application is routinely practised. Control of WBD by pruning of brooms has been found to be partially effective, though costly in labour. Removal of sources of inoculum in this way must also be applied by all farmers in a given area to be effective. Field trials at the Nestlé Research farm in Ecuador have shown this method to be effective for the control of WBD, but only when trees are kept small (<2 m high) and battery-powered secateurs are used to reduce pruning costs (C. Ruales pers. comm.). Removal of infected pods is also effective against FPD, though the disease is only manifest shortly before the pathogen sporulates (Soberanis et al. 1999).

The ideal solution to the problems of both WBD and FPD 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 WBD resistant cultivars (Pound 1940; Allen 1987), 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 (cacao is open-pollinated and self-incompatible) but, despite its tolerance of WBD, it lacks the prized Arriba flavour of more traditional Ecuadorian varieties (Anon. 2002). Similarly in Brazil, several clones with elevated resistance have been developed in recent years. Combined with advances in somatic embryogenesis and micropropagation techniques, it is possible to deploy these resistant clones much more rapidly than previously (Guiltinan et al. 1997). However, it remains to be seen how effectively these clones will withstand the disease in the field. The fact that strains of the C-biotype of *C. perniciosa* show little genetic diversity, combined with the pathogen's long life cycle (c. 12 months), does hold out some hope that, once identified and disseminated, resistant genotypes will prove stable in the field.

A new weapon in the pathologist's armoury against WBD and FPD is biological control (Krauss & Soberanis 2001). For example, *Trichoderma stromaticum* (Hypocreales) is an effective hyperparasite of the mycelium and basidiocarps of *C. perniciosa* (Sanogo et al. 2002). Trials are currently under way 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 fungi to protect against WBD and FPD. A range of fungi inhabit the internal tissues of cacao meristems (Arnold et al. 2001). Although these infections are asymptomatic, research is in progress to assess whether the presence of endophytes in meristematic tissues can inhibit establishment and spread of *C. perniciosa* and *M. roreri* (http://www.oardc.ohio-state.edu/cocoa/sustain.htm).

CONCLUSION

Clearly, we have much to learn, not only about the evolutionary biology and breeding strategies of these pathogens but also about the processes of pathogenesis. One consequence of the most recent WBD outbreak in Bahia is that several Brazilian research groups are now directing their efforts towards elucidating aspects of the biology of this fungus. Most notably, a *C. perniciosa* genome sequencing programme is now well under way, co-ordinated by Prof. G. Pereira at UNICAMP in Sao Paolo Province and involving several satellite labs (http://www.lge.ibi.unicamp.br/vassoura/). It is anticipated that this programme will approach completion in 2003. This will represent a significant technological milestone for Southern Hemisphere biology; it will be the among first agaric fungi to be fully sequenced, and the first eukaryote to be sequenced by a Southern Hemisphere nation. It is to be hoped that this sequence information will provide insight into the biology of this fungus and indicate mechanisms whereby WBD can be controlled.

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