Cacao Diseases in the Americas: Myths and Misnomers

Harry C. Evans
CAB International, Egham, Surrey, UK

The catalyst for this article was a recent report in FUNGI relating to a fungal foray in Amazonian Ecuador (Evans and Winkler, 2011). Passing mention was made of the fungi attacking cacao pods: "The main pathogen is *Monilia*, a powdery mildew fungus that is spread by insects." Such a confusion of both mycological and pathological facts only adds to the continuing myths and misnomers surrounding this disease (frosty pod rot), as well as to the more infamous witches' broom disease, and the causal agents. This warranted a response and a clarification of the status of two fungi which have changed the history of cacao cultivation in the Americas, along with the inevitable and incalculable socio-economic impacts. Indeed, they could play an even more pivotal role on the global commodity-market stage should these pathogens ever escape their Neotropical shackles.

Early History of Cacao

Cacao is unique amongst the world's crops in that it was never cultivated in its center of origin or diversity–the Upper Amazon basin of South America. Precisely how and when it reached Mesoamerica remains a mystery, but recent chemical and archaeological evidence indicates that it was being consumed, and enjoyed, at least 3,000 years ago in present-day Honduras (Henderson et al., 2007). However, it was in the Yucatan region of Mexico that it reached its peak of importance becoming a unit of currency for the Mayans, as well as for the Aztecs to the north. Thus, the plant escaped its coevolved natural enemies and obviously thrived in these pest-free situations. Indeed, fructuous spontaneous populations can still be found in the Mayan relic-rich forests straddling the Guatemalan-Mexican border where "Criollo" cacao has become a naturalised understory tree (Author, pers. obs., 1999). It was here that the myth of two centres of evolution of cacao arose–now finally debunked thanks to DNA fingerprinting (Motamayor et al., 2008)–which even spawned the idea that an endemic subspecies had evolved there; depicted in Mayan reliefs as vine-like plants (Thorold, 1975).

After the conquest of Mexico, the Spanish were quick to appreciate the commercial and culinary value of cacao and introduced it into Europe and, by the 17th century, fashionable chocolate houses–the forerunners of gentleman's clubs–were springing up in London. However, the product was not as we know chocolate today, and it was not until the 19th century that the process of extracting cacao butter and adding milk was perfected and, as they say, the rest is history (Grivetti and Shapiro, 2009). To meet the rapidly-growing demand for chocolate products, commercial cultivation took off for the first time in the native range of cacao and its relatives, in northern South America. It was only a matter of time before coevolved fungal pathogens moved–naturally or human assisted–from their forest hosts into the cacao plantations. witches' broom was the first of these fungal diseases to be investigated scientifically.

Witches' Broom

The evocatively named genus *Theobroma* ("food of the gods")–of which cacao is the best known member –is a common representative in Amazonian forests where the main disease is witches' broom, so-called because of the grossly distorted, hypertrophied shoots. In wild cacao, however, the disease is often cryptic, typically, manifested only by malformed flower cushions producing grossly misshapen pods; whereas, in sharp contrast, its close relative *Theobroma grandiflorum* ("cupuaçu")–highly prized in the Brazilian State of Pará for its aromatic pulp surrounding the beans–is often severely attacked (Figs. 1-2), so much so that it is known locally as "a mae da vassoura-de-bruxa" (the mother of witches' broom). There are anecdotal reports that Portuguese explorers in the 18th century had reported the disease under the name

Figure 1. Vegetative witches' broom on *Theobroma grandiflorum* (cupuaçu) in the Brazilian Amazon.

Figure 2. Witches' broom infection of flower cushions of *Theobroma grandiflorum*, resulting in malformed flowers and young pods.

Figure 3. Pod lesion caused by witches' broom, developing 2-3 months after infection of young pod, accompanied by characteristic premature ripening and invasion by opportunistic fungi, predominantly *Colletotrichum* and *Fusarium*. Secondary colonization of both necrosing pods and brooms led to the early myths and misnomers concerning the causal agent. Fruit-bodies of the real pathogen may not appear for another 6-12 months.

"lagartão" (Silva, 1987), presumably referring to the lizard-like appearance of the green swollen brooms in the tree canopy. However, the first confirmed description of the disease was from the late 19th century in Suriname where it received its Germanic "krülloten" common name, initially translated as witch-broom (Van Hall and Drost, 1909). These authors detailed the early attempts to identify the causal agent of the disease which at that time was sweeping through the previously lucrative cacao plantations, drastically reducing yield - since it attacks not only vegetative and reproductive shoots, but also pods - and which they finally attributed to a new species of *Colletotrichum*, *C. luxificum* (Fig. 3). This diagnosis was accepted by notable European mycologists, such as G. Massee, who included a full description of the "pathogen" in his plant disease book (Massee, 1910). Earlier, preserved broom specimens sent to Dutch mycologists had yielded several possible causal agents, including *Exoascus theobromae*, described by J. Ritzema Bos in the early 1900s, *Fusarium* and *Lasiodiplodia.* Later, J. B. Rorer, an American mycologist based in Trinidad, consistently isolated mycelial cultures bearing clamp connections from green brooms, and concluded that the pathogen must be a Basidiomycete; although he failed to achieve infection. Finally, 25 years after the first scientific identification of the disease, the Swiss mycologist G. Stahel, working in Suriname, identified and named the pathogen as *Marasmius perniciosus* (Stahel, 1915): a novel basidiomycete species producing small, crimson mushrooms on necrotic brooms (Figs. 4-6).

Almost three decades later, R. Singer transferred it to *Crinipellis*, as *C. perniciosa* within the section Eu-

Figure 4. Fruit bodies of the witches' broom pathogen: young bell-shaped basidiomata, showing centrally–aggregated crimson hairs on the pileus (ca. 1 cm diam).

Figure 5. Basidioma expanding and becoming pinkish with age, with release of basidiospores. Note white spore shadow below and compare with sporulating capacity of the frosty pod rot pathogen (Figs. 8-9,13-14).

Crinipellis, sub-section Iopodinae (Singer, 1942). Much later, Singer (1976) recognized Iopodinae as a separate section, placing *C. perniciosa* in the new sub-section Insignes. Pithily, and somewhat prophetically, he noted: "The fact that the fungus is still frequently quoted as *Marasmius perniciosus*, 30 years after its transfer to *Crinipellis*, is a good illustration of the 'conservatism' of some phytopathologists and their reluctance to adopt the results of mycological work." There have been

Figure 6. View of basidioma from below, showing stipe and gill structure.

Figure 9. Detail of symptoms of frosty pod rot on maturing cacao pod, showing the white pseudostroma on which the slightly pigmented spores are produced, imparting a creamish brown color to the initial frosted appearance.

Figure 7. Witches' broom on solanaceous host, *Solanum mauritianum*, in Minas Gerais, Brazil.

Figure 8. Late symptoms of frosty pod rot of cacao, demonstrating the prolific spore production and the ready release of spore clouds, dispersed by convection currents, negating the long-held myth that the pathogen is insect vectored.

attempts since to subdivide the species–based mainly on pigmentation of the pileus (e.g. Pegler, 1978), as well as on the discovery that other plant families are also hosts of this fungus (Fig. 7; Evans, 2007)–but these have not met with general acceptance. Here, the story stalled until the advent of the molecular era.

Frosty Pod Rot

One of the lesser known diseases in the plant pathology world, frosty pod rot of cacao has continued to fascinate and frustrate those who have come into contact with it over the past 100 years. The first scientific encounter with the disease was probably by C. J. J. Van Hall who visited western Ecuador during the cacao boom years in the early 1900s and reported on problems affecting the crop, including a condition known locally as "helada" (frost)–"producing abnormal growth of the pods and beans" (Van Hall, 1914)–and popularly considered to be the result of unfavorably low temperatures. It seems probable that: either, he never actually witnessed the disease in the field; or, he visited during the long dry, inter-crop period, since he was sufficiently experienced to have identified the disease as fungal in origin due to the conspicuous spore masses produced on and released from infected pods (Fig. 8). It was left, once again, to J. B. Rorer–invited from Trinidad by the cacao "barons" in 1917 to investigate the mysterious pod malady making severe inroads into the considerable wealth generated from their vast estates – to recognise the precise fungal nature of the problem. Rorer sought the help of specialists in the USA and the causal agent was duly identified as a *Monilia* sp., close to *M. fructigena*, with obvious analogies made to this temperate pathogen, such as likening the mummified cacao pod to a sclerotium. Nearly two decades elapsed before the fungus was named in his honor, *Monilia roreri*, by the versatile and prolific Italian mycologist R. Ciferri, when material was despatched to him from Ecuador (Ciferri and Parodi, 1933). He also coined the common name "Moniliasi," which has persisted in one form or another in Latin America and the cacao literature, in general, ever since.

Over 40 years later, the aetiology of the disease was investigated in more depth–in particular, using modern techniques of microscopy (SEM, TEM) to elucidate sporogenesis and hyphal ultrastructure–leading to the conclusion that the causal fungus is the asexual form (anamorph) of an unknown Basidiomycete and to the erection of the new hyphomycete genus *Moniliophthora*: literally, "*Monilia*-destroyer" (Evans et al., 1978). In a further bid to eradicate the association with *Monilia*, a new common name, frosty pod rot of cacao was proposed, based on the various Spanish vernacular names that accurately describe the frosted appearance of the pods (Figs.

Figure 10. Typical swollen, convoluted, monokaryotic, parasitic mycelium growing intercellularly within cacao pod infected by *M. roreri*: this biotrophic phase is similar to that in pods infected by *C. perniciosa* and can persist for 2-3 months before external symptoms appear.

Figure 11. Prematurely maturing, seemingly healthy cacao pod with "green islands," indicating systemic infection by a biotrophic pathogen.

8-9, 13-14; compare Fig. 3). It has not worked at either end of the scientific spectrum: field technicians still refer to it as "la Monilia" or "Moniliasis" (but, not many farmers, who use the local descriptive names); whilst, in higher scientific echelons the myths and misnomers continue unabated. G.N. Agrios, for example, in his classic book on plant pathology has painted a confused picture over the years. In the penultimate edition (Agrios, 1997), the pathogen is placed in the Sclerotiniaceae: Ascomycota, as "Monilia pod rot of cacao caused by *Monilia roreri*;" whilst, in the latest edition (Agrios, 2005), it still remains in the Sclerotiniaceae but with the mixed message–"Monilia pod rot of cacao, caused by the fungus *Moniliophthora roreri*, anamorph *Monilia roreri*." This may have been influenced by the erroneous entry in the *Dictionary of the Fungi* (Kirk et al., 2001), where it is described as "anamorphic Ascomycetes (synanamorph *Monilia roreri*)." More unfortunately, however, the chosen images of the disease–received (in good faith, presumably) from an international agricultural institute in Nigeria (on a continent where the disease is absent!), show an unconvincing early stage in infection and, purportedly, a group of severely infected cacao pods. Unequivocally, the latter are pawpaw fruits (*Carica papaya*) covered with a mildew-like growth.

Post-Molecular Era

As reported above for cacao (Matamoros et al., 2008), molecular techniques have helped to dispel most of the myths and misnomers that have obscured the true relationships and origins of these two cacao pathogens. In the pre-molecular era, similarities had been drawn between the fungi based on pod symptoms, especially the morphology and physiology of the endophytic (biotrophic) parasitic phase (Figs. 10-12): "Until more evidence is available, it is tentatively suggested that *M. roreri* and *C. perniciosa* have a common origin" (Evans, 1981). This seemingly speculative statement was confirmed 20 years later when their ITS and small mitochondrial rDNA sequences were found to be closely matched, which prompted the proposal that *M. roreri* should be transferred to the genus *Crinipellis* (Evans et al., 2002). Subsequently, frosty pod rot was discovered on *Theobroma gileri*, a rare indigenous understory tree in remnant submontane forest on the north-western slopes of the Ecuadorian Andes (Fig. 13), and the new combination *Crinipellis roreri* was formally made (Evans et al., 2003a). It seemed that the purported endemic host of the cacao pathogen had finally been tracked down (Evans, 1981; Evans, 2002). However, when the isolate from *T. gileri* was tested for pathogenicity to cacao, none of the inoculated pods became infected (Fig. 14). A closer examination of spore morphology and DNA sequences revealed significant differences between the two strains and the new taxon *C. roreri* var. *gileri* was erected (Evans et al., 2003b). Thus, the mystery remains as to the origin of the cacao pathogen, var. *roreri*. It appears that it entered Ecuador during the height of the cacao boom years - almost certainly through human agency–possibly, via cryptically-infected pods imported as cacao germplasm. Cacao has an ancient history of cultivation in northern Colombia (Thorold, 1975), and an endemic species of *Theobroma*, similar to *T. gileri*, has been reported in this region (Cuatrecasas, 1964; B.D. Bartley, pers. comm.). It is posited that over the centuries of contact between the forest host and cacao, the fungus adapted to this new, high-density host.

Recently, more comprehensive phylogenetic analyses, using five gene regions, have provided irrefutable evidence that the two cacao fungi represent sister species in the Marasmiaceae (Aime and Phillips-Mora, 2005). However, they form a distinct lineage within the family and the new monophyletic clade separates readily from all other genera. Thus, the cacao pathogens could not remain in the genus *Crinipellis* and the authors opted to retain the name *Moniliophthora* rather than erect a new genus to accommodate them. The new combination *Moniliophthora perniciosa* was made; although, a re-description of this previously anamorphic genus to include species characterised by mushroom fruitbodies was not deemed necessary.

Further Thoughts

This is an ideal forum for indulging in further, speculative thoughts without upsetting too many scientific apple carts! In terms of taxonomy, both cacao pathogens are sister species in a genus that is probably widespread in tropical America but one which, essentially, is endophytic in habit: the majority of its taxa are probably living in cryptic, symbiotic relationships with their host plants. It is also probable that all the species included in the subsection *Insignes* of the genus *Crinipellis* by Singer (1976) belong here, and basidiomata of several of these species (e.g. *C. eggersii, C. siparunae)* have been observed in Amazonian forests emerging en masse from the trunks of healthy trees (Author, pers. obs.). So, why did the two species associated with cacao and its relatives "turn the tables" on their hosts and become parasitic, altering the growth patterns of the colonised tissues for their own benefit and, ultimately, causing their deaths?

Studies, currently underway in Brazil, to test the cross-infectivity of *M. perniciosa* isolates from a range of unrelated hosts may, serendipitously, have answered this question following a series of unexpected and highly abnormal infection events. There is now compelling, albeit circumstantial evidence of a complex tri-trophic relationship in action and that a third, cryptic partner is the true causal agent of the disease. The fungus acts merely as an endophytic vector, carrying infectious particles into the susceptible plant species without triggering the host defence reactions: this agent then alters the hormonal balance, resulting in abnormal meristematic activity (H.C. Evans and R.W. Barreto, unpublished data). It is posited that a benign, endophytic progenitor of *M. perniciosa* acquired this infectious particle many millennia ago and, over time, extended its host range to include common understory forest species in at least four plant families: Bignoniaceae, Malpighiaceae, Malvaceae and Solanaceae, whilst extending its geographic range to reach as far as Minas Gerais in southeast Brazil (Evans, 2007; see Fig. 7). These strains gradually

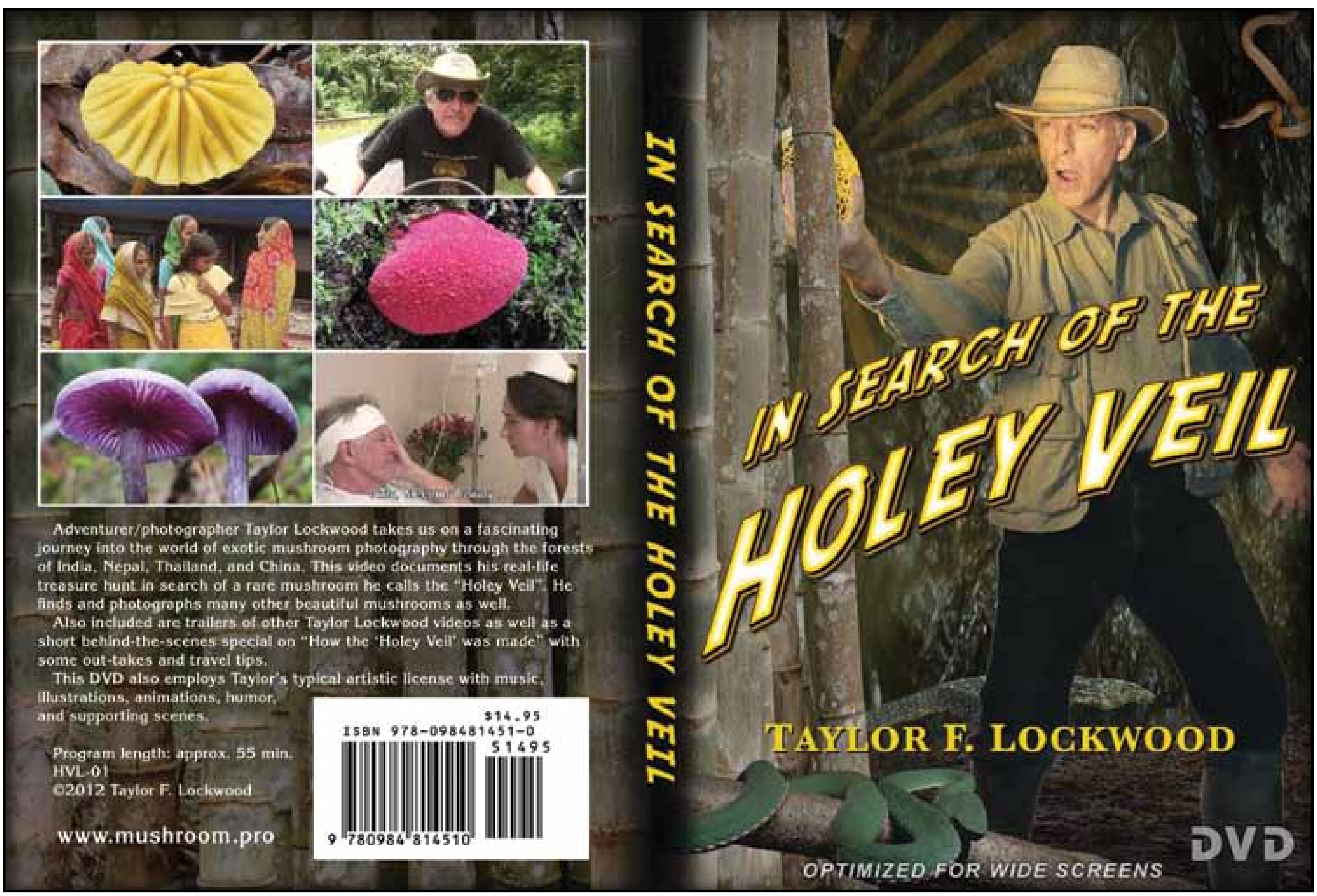

Figure 12. Section through pod in Fig. 11 showing abnormal development and destruction of internal tissues by *M.roreri*, with watery necrosis as the fungus switches to the saprophytic phase, 2-3 months after initial infection. At this stage, pod symptoms are identical to those caused by witches' broom disease.

Figure 14. Experiment which proved that the frosty pod rot pathogen from the indigenous tree, *Theobroma gileri*, was not responsible for the disease epidemics in cacao plantations in western Ecuador in the early 1900s. The upper row of harvested healthy pods was inoculated, 3 months previously, with the fungus from *T. gileri*; the lower row of "frosted" pods was inoculated with the cacao pathogen.

Figure 13. Pod of *Theobroma gileri* infected with *M. roreri* in submontane forest in NW Ecuador.

evolved into species-, genus- or family-specific pathotypes. But, where does the sister species *M. roreri* fit into this hypothetical scenario?

With the final upsurge of the Northern Andean Cordillera - and the establishment of the current Amazonian drainage system, some 3 million years ago (Ribas et al., 2012)–the western populations of the fungus became genetically isolated from the mainstream populations on their much more ubiquitous hosts in the Amazon basin. In sharp contrast, malvaceous hosts in the genera *Herrania* and *Theobroma* are restricted to only a few species scattered through the coastal and submontane forests of northwest South America (Cautrecasas, 1964; Evans, 2002 and pers. obs.). Low host density, therefore, became the evolutionary pressure to increase both spore survival as well as spore production. The interpretation of subsequent events presented here, is that the entire pod has been transformed by the fungus into a giant "basidioma" producing billions of long-lived, efficiently-dispersed infective spores from an external pseudostroma (Evans, 1981). Cytological evidence indicates that these are true meiospores (Evans et al., 2002; Evans et al., 2003b), implying that the supposed conidiophores, in fact, represent modified basidia. There is no new mushroom waiting to be discovered and described: the author having spent several "fruitless" years in Ecuador trying to track it down.

In summary, most but not all of the myths and misnomers have been or are being addressed. However, there remain lingering doubts on whether or not the original "anamorphic" genus *Moniliophthora* is a fitting resting place for these extraordinary fungi.

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Coprinus comatus. Photo courtesy of Jan Hammond.