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GENETIC DIVERSITY IN WITCHES' BROOM DISEASE

Improving our knowledge of the genetic diversity of the fungi which cause Witches' Broom Disease and Frosty Pod Rot

Witches' Broom Disease of cocoa is a significant factor affecting productivity of cocoa in parts of South America and the Caribbean. When this project was initiated the causal fungus was known as *Crinipellis perniciosa*, though some similarities with the fungus causing Frosty Pod Rot, *Moniliophthora roreri*, had long been noted. This study contributed to the body of molecular evidence for the close phylogenetic relationship between the two organisms which led to the re-naming of *C. perniciosa* to *Moniliophthora perniciosa* in 2005.

M. perniciosa is a basidiomycete fungus which has a complex and unusual lifecycle which involves initial biotrophic infection within living host tissues, stimulating the production of characteristic green "brooms", followed by a saprotrophic phase synchronous with the death of the plant tissues. 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. This study investigated the genetic diversity of a range of over 160 isolates collected from various sources and maintained at Aberystwyth University together with some isolates of *Crinipellis* species and *M. roreri*.

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Phylogenetic analysis of several regions of the ribosomal RNA (rRNA) locus was undertaken but very little variation was detected between geographically distinct isolates of the C- and S-biotypes. This confirms previous work with isozymes that also found that the groups are very closely related and suggests that the C-biotype evolved from the S-biotype very recently. Some genetic polymorphism was found between C-biotype isolates using AFLP analysis but it was not possible to use the information to gain an insight as to the centre of origin of the disease or its spread to the cocoa growing area of Bahia from the Amazonian region, though it seems likely that this was through human intervention. The L- and H-biotypes formed separate groupings, with the former showing considerable genetic variability with sequence polymorphisms present between isolates that were collected only a few metres apart in the field. Preliminary analysis showed that the Witches' Broom and Frosty Pod isolates were more closely related to each other than to any other members of the *Crinipellis* genus. Attempts to form hybrids between the two were unsuccessful though some degree of hyphal compatibility was observed.

Work at Aberystwyth has also aimed to better understand the process of host infection. In contrast to earlier work by Frias, which found that host penetration was mostly via stomata, our work suggests that collapsed trichome bases on young meristems (where stomatal density is low) may be an important route for pathogen entry.

Publications:

Griffith, GW, Nicholson, J, Nenninger, A, Birch, RN & Hedger, JN. (2003). Witches' brooms and frosty pods: two major pathogens of cacao. *New Zealand Journal of Botany*, 41(3), 423-435.
Artero, AS., Silva, JQ., Albuquerque, PSB., Bressan, EA, Leal Jr, GA., Sebbenn, AM, Griffith, GW, Figueira, A, (2016). Spatial genetic structure and dispersal of the cacao pathogen *Moniliophthora perniciosa* in the Brazilian Amazon. *Plant Pathology* 66, 912-923.

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