

A West Africa-wide survey of CSSV, its alternative hosts and vectors

Using molecular markers to investigate the distribution of Cacao Swollen Shoot Virus and developing an identification technique for the mealybug vectors

Cocoa Swollen Shoot Virus Disease (CSSVD) has caused substantial losses to cocoa production through much of West Africa. Control of the disease has been severely hampered by a lack of information about the distribution, movement and strain profile of the virus and the mealybugs which carry it. Without this knowledge it is difficult to determine an appropriate management strategy and inform farmers of the steps they need to take to protect their cocoa. Moreover, although it is known that at least 15 species of mealybugs can act as vectors, each with different mobility and transmission characteristics which affect their ability to spread the virus, they are extremely difficult to identify by eye. This research project studied the distribution of viral strains across West Africa through collaboration with the research institutes in the region and developed a DNA barcoding technique which allows identification of mealybug species, even at the egg and youngest instar stages of growth. A set of Universal PCR Primers, developed during a previous CR(UK) supported project, was used to analyse leaf samples from 1641 GPS tagged sites in Côte d'Ivoire, Ghana and Nigeria. CSSV was detected in cocoa leaves from 12% of the sites, from 5°57'E (Uhomora, Nigeria) to 6°48'W (Pélési, Côte d'Ivoire) and from 4°45'N to 7°29'N (Bahi and Adedokun, Nigeria respectively). A wide range of putative host plants of CSSV growing at these sites

were also screened. Generally only members of the Malvaceae exhibited CSSV sequences, the most frequent being *Ceiba pentandra*.

Mealybugs were sampled from cocoa trees in South America, the Caribbean and SE Asia, as well as from multiple sites in Ghana, Côte d'Ivoire and Nigeria. The species were identified using Environmental Scanning Electron Microscopy with the help of an expert entomologist and then DNA was extracted from the individual samples. DNA barcodes generated using cytochrome c oxidase-based PCR primers were able to distinguish the 11 different reference species. A High Resolution Melt analysis system was also established which enables the rapid identification of an unknown mealybug without the need for access to DNA sequencing. *Planococcoides njalensis* (Laing) and *Planococcus citri* (Risso) were the most frequently detected mealybug species among those collected from cacao throughout West Africa. Both are known to be key vectors of CSSV.

Publications:

Wetten,A.C, Campbell, C.A.M. and Allainguillaume, J. (2015) High-resolution melt and morphological analyses of mealybugs (Hemiptera: Pseudococcidae) from cacao: tools for the control of Cacao swollen shoot virus spread. *Pest Manag Sci* (2015). DOI 10.1002/ps.4017

Wetten,A.C, Campbell,C.A.M. and Allainguillaume, J. (2012). A PCR-based approach for the identification of Cacao Swollen Shoot Virus mealybug vectors. Proceedings of the 17th International Cocoa Research Conference, Yaounde, Cameroon. COPAL.

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For more information please contact:

Dr. Andy Wetten, formerly of School of Agriculture, Policy and Development, University of Reading, UK, now at University of the West of England ☎ andy.wetten@uwe.ac.uk

