

## M E M O R A N D U M

Your Ref:  
Our Ref:

Date: 2 March 1992

FILE NOTE

From: Rob Lockwood

SUBJECT: SUSPECTED COCOA VIRUS IN MALAYSIA

1 BACKGROUND

1.1 In September 1991 a "fern leaf" symptom was reported on introduced clone GS50 growing at four locations in Sabah. The clone, which is of Caribbean origin, passed intermediate quarantine in Miami, Florida and post-entry quarantine at Highlands Research Unit (HRU) in Peninsular Malaysia. Budwood was taken from HRU for further quarantine at Tuaran in Sabah. The clone was one of a batch of 31 imported by the Malaysian Agricultural Research and Development Institute (MARDI) and Malaysian Cocoa Growers' Council (MCGC) in 1986.

1.2 The symptoms on GS50 are similar to these seen on imported clones in Sabah in the late sixties. The cause then was shown to be graft transmissible and it was inferred that a mild virus had passed intermediate quarantine at Kew. However, the same symptoms were seen on clones imported directly from Indonesia, where virus infection has been reported for many years.

1.3 In Ghana in 1971-75 a fern leaf symptom on clone DR38 from Indonesia was shown to be associated with a mealybug - transmissible agent. Infection was demonstrated in DR1 by transmission tests, but symptoms were not observed over a five year period. DR1 and DR38 passed through intermediate quarantine at Wageningen.

1.4 In 1981 a mealybug transmitted virus with the same particle shape and size as cocoa swollen shoot virus (CSSV) was recovered from cocoa growing in Sumatra.

1.5 Transmission tests with GS50 are in progress in Sabah.

1.6 Dr Mike Thresh thought that leaves of GS50 sent to England had symptoms highly suggestive of virus infection.

1.7 Leaf extracts from GS50 have been tested against antisera to Ghanaian strains of CSSV at both Scottish Crops Research Institute (SCRI) and John Innes Institute (JII) using the ELISA technique. The results were negative, but ELISA tests for CSSV are known to be highly specific to the virus strain, and will not pick up virus in samples from which the symptoms have disappeared.

1.8 At SCRI leaf sections of GS50 were examined by electron microscopy. No particles were seen, but as previous experience is that the virus titre for CSSV is very low, there is only a remote chance of seeing particles directly.

1.9 At JII partially purified extracts were tested against three experimental DNA probes for CSSV. Two reacted positively. However, the technology has not been proven for mature leaves and the result is not considered to be completely conclusive.

1.10 At JII the same extracts were examined by electron microscopy. Again no particles were seen.

1.11 In Sabah all field grown trees of GS50 were cut out in September 1991. JA546 from the same batch was also cut out although it showed symptoms only at Tuaran. Photographs indicated a clear fern leaf pattern. In November C87-56 and SPEC138-9 were cut out, although no clear symptoms had been seen. When trees were removed, one row of contact trees was removed with them.

## 2 PENINSULAR MALAYSIA

2.1 GS50 and the other 30 clones in the batch were distributed to some, but not all MCGC members. It is known to be growing at Sime Darby's Merlimau Estate, IOI's Jasin Lalang, Golden Hope's Sungei Wangi and FELDA Tun Razak research station. It is not at MARDI or United Plantations.

2.2 All mature trees of GS50 at FELDA were said to be showing symptoms and all except one had been cut out. The remaining one showed an inconclusive fern leaf pattern.

2.3 At Jasin Lalang and Merlimau almost every tree showed clear symptoms. There was insufficient time to visit Sungei Wangi, where GS50 was said to be free of symptoms. It is now thought that the wrong symptoms were sought.

2.4 The only symptom seen was a more or less distinct fern leaf pattern in mature leaves. There was no vein banding, vein clearing or other symptom in flush leaves. Swellings were not seen, but they are characteristic of a limited number of CSSV strains. Symptom - bearing trees were growing and fruiting normally.

2.5 The fern leaf symptom was not found on JA5.46, C87-56 and SPEC138-9.

## 3 FURTHER INVESTIGATION

3.1 Inspect GS50 and the other three clones at other locations.

3.2 Initiate regular inspection of the other 27 clones in the batch.

3.3 GS50 budwood from FELDA, Jasin Lalang and Merlimau was sent to Reading University to be grown on outside the quarantine area and provide material for further investigation.

3.4 Mature and flush leaves of GS50 from FELDA, Jasin Lalang and Merlimau were sent to JII, together with mature and flush leaves from supposedly healthy mature seedling trees at Merlimau.

3.5 Ong Chin Yang of MARDI Serdang will attempt to obtain partially purified virus from flush leaves at Jasin Lalang and Merlimau. Preparations will be used for electron microscopy and could be sent to JII for probe tests. If JII supplies the probe, tests can be done at Serdang, where ELISA technology is available too.

3.6 If GS50 is shown to be infected, attempts should be made to find out where infection occurred.

#### 4 CONTROL

4.1 If a virus of the CSSV type is present it is likely to be vectored by a limited range of mealy bug species. It is sufficient to cut out trees with symptoms and one row of contacts. Main timbers can be stacked to dry out and trash heaped into slash piles and left to rot. There is no need to burn.

4.2 It would be unwise to propagate any of the other 30 clones except for supplying the germplasm collection.

4.3 There is no known risk of pollen or seed transmission so breeding work including pollination and seed production can continue.

#### 5 IMPLICATIONS

5.1 The inference that GS50 is infected with a virus compromises the Mayaguez clone collection only if it is confirmed that it is infected at Mayaguez and it has been grown in the field with other clones.

5.2 If GS50 was infected with a virus when it left Mayaguez it will be the second or third time that a virus has passed undetected through intermediate quarantine. It can be argued that the failure rate is unacceptably high and that procedures must be changed. At present there is no alternative to a protracted period of observation on both the clone and an indicator such as seedling West African Amelonado, but experience has shown that this may not be wholly reliable. Plants grown in pots for long periods commonly develop symptoms of nutrient imbalance which may mask any due to virus

infection. With probes, the sensitivity and strain specificity remain to be established. This highlights the need for a concentrated effort on the development of rapid and reliable (if possible) diagnostic techniques. There is need for dialogue between the quarantine stations and receiving countries as to what procedures are both practicable and reasonably effective.

#### 6 FURTHER REPORTING

A record of results of investigations at John Innes Institute will be circulated when they are complete.

#### 7 ACKNOWLEDGEMENTS

7.1 Congratulations are due to Mr Yip Kin San, State Quarantine Officer in Sabah, who spotted the fern leaf symptom in GS50.

7.2 Cik Sapiyah is thanked for facilitating visits to MARDI, United Plantations and FELDA and Mr Ooi Ling Hoak for arranging visits to Merlimau and Jasin Lalang Estates, Mr Thong Fah Thor kindly provided office facilities.

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GL/HL  
cocoa. mem

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