Cocoa Quarantine Project

Department of Horticulture
School of Plant Sciences
University of Reading

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Introduction

We are continuing to develop the cocoa quarantine facilities here at Reading to meet both the requirement for a longer quarantine period (see below) and the increased demand for safe germplasm exchange. Our workload has continued to increase, especially since the loss of the USDA Miami quarantine facility in the hurricane of 1992. We have now added a third span to the greenhouse so that the available floor space for quarantine operations has been increased by 50%. The greenhouse is divided into three separate compartments which allows better isolation of incoming material, material being virus-indexed and fully quarantined material. In addition we have increased our seedling raising capability through the use of a second greenhouse. However, space is still very limited, especially in our compartment for fully quarantined material which currently contains over 120 large plants. Information from the International Cocoa Germplasm Database and from our own records of budwood export suggests that many of the 70 clones in this compartment have already been widely distributed and we plan to repropagate them and maintain much smaller plants. Of course this will mean that the budwood available from these clones will be very limited for a period of time after repropagation. It is very important, therefore, that anyone interested in obtaining budwood from these clones should notify us as soon as possible.

Cocoa Quarantine Management Committee

Since our last newsletter a Cocoa Quarantine Management Committee has been formed to oversee and advise on quarantine operations. The terms of reference of the committee are:

(1) To determine the policy of operations of the quarantine facilities at Reading University.

(2) To ensure the procedures minimise risk of the dissemination of pests and diseases.

(3) To determine the material to be quarantined (with requirements of cocoa breeders/contributors in mind).

(4) To ensure that operation of the facilities is conducted in a cost effective way commensurate with points (1) - (3) above.

Current membership of the Committee is as follows:

Mr. R.A Lass	-Chairman
Mr. E.T. Beauchamp	-BCCCA
Dr. Michelle J. End	-Reading University
Dr. P. Hadley	-Reading University
Dr. Angela Isaac	-NRI (Virologist)
Dr. G. Lockwood	-CDC (Plant Breeder)
Dr. J.M. Thresh	-NRI (Virology Consultant)
Dr. B.E.J. Wheeler	-Imperial College (Pathology Consultant)
Mrs Teresa Wheeler	-Reading University

On occasions other interested parties attend the meetings as observers.

Entomology, Pathology and Virology Consultants

Prof. H.F. van Emden, Dr. Bryan Wheeler and Dr. Mike Thresh/Dr. Angela Isaac have agreed to act as entomology, pathology consultant and virology consultants, respectively, to the quarantine facility. They will make periodic visits to check that our procedures are appropriate, to inspect material in quarantine and give technical advice as required.

Tissue Culture

There has been considerable interest internationally in developing a reliable system for cocoa micropropagation. We are particularly

interested in such techniques as they offer a rapid and convenient means of distributing quarantined plant material to cocoa producing countries. A technique of producing plants from cultured cocoa buds has recently been published from work carried out at Pennsylvania State University (Flynn *et al.*, 1990) and we have been assessing their published technique. Although, inevitably, we have not been able to commit a great deal of time to this, we have been able to obtain both root and shoot proliferation from cultured buds and believe that their technique shows great promise. As yet we have not been able to produce plantlets but we hope to achieve this in the near future. Encouraging reports on successful cocoa micropropagation techniques were reported by workers from Purdue University, USA, MARDI, Malaysia and CATIE, Costa Rica at the International Cocoa Conference held in Kuala Lumpur and by workers from Purdue University (USA) and Francereco-CIRAD (France) at the 11th International Cocoa Research Conference in the Côte d'Ivoire.

New Techniques in Virus Indexing Cocoa

Much of the research on virus detection in cocoa has been concentrated on the detection of Cocoa Swollen Shoot Virus (CSSV), a member of the Badna virus group. The current recommended method is via visual inspection from virus transmission from buds grafted to West African Amelonado, a type of cocoa that produces characteristic leaf symptoms when infected with CSSV. This protocol has been used by quarantine stations for 30 years although the length of the test period has recently been extended from 3 flushes to two years following a recommendation made at the Workshop on the Conservation, Characterization and Utilization of Cocoa Germplasm, Trinidad in 1992. The disadvantages of this test are the long inspection period and the possibility that new or unknown viruses will not produce recognizable symptoms in the Amelonado test plants.

It would be highly desirable to reduce the length of the quarantine period and improve the reliability of virus detection. Several areas are being actively researched in to the detection of viruses and viroids in cocoa and other species. These include mechanical transmission to herbaceous indicator species, examination by electron microscopy, immunological techniques and the use of nucleic acid probes. An immunological technique (enzyme-linked immunosorbent assay ELISA) was developed by Sagemann *et al.* (1985) which can be used to confirm the presence of CSSV, but this can only be used if leaf symptoms are present. Research has shown that the various strains of CSSV are genetically quite different, indeed, it is not clear whether CSSV represents one highly variable virus, or more than one virus that come from different original hosts. Current research suggests that different anti-sera will be needed to detect the various strains. A promising new technique for CSSV detection, the Virobacterial Agglutination (VBA) test, is currently under development at CRIG (Ghana) (Hughes & Ollenu, 1993). This technique can be used to detect both mild and severe strains of CSSV even where no visible symptoms are present. CRIG are continuing to develop anti-sera which can be used to detect a wide range of CSSV strains and we have recently started a project in collaboration with CRIG to investigate the possibility of using this test routinely as part of our quarantine operation.

It is possible that new molecular techniques may be able to provide a detection method for a whole range of different viruses. Progress is being made in the detection of viral nucleic acids, such as double-stranded RNA, which are not normally found in plant tissue. However, research is still needed to assess the feasibility of such techniques in cocoa. Another possibility is the development of nucleic acid probes which when used in conjunction with the polymerase chain reaction (PCR) have great potential in detecting viral DNA even if it is only present in very low concentrations. At present probes have been produced which can be used in strain specific tests, but it is hoped that a probe can be developed which can be used to detect a much wider range of virus types.

Cocoa Budwood Exports

We are still anxious that if people interested in the transfer of germplasm, or their colleagues, are visiting the UK that the opportunity is taken of returning with cocoa germplasm. This is by far the easiest and most successful means of transporting material from the collection. It is also possible to arrange for material to be carried by a courier company such as DHL. Transport by such a courier costs in the region of £50 and delivery normally takes less than 5 days which means that the budwood arrives in reasonably good condition.

Required Documentation for Exporting Cocoa Germplasm from the UK.

We would like to take the opportunity to remind those requesting cocoa germplasm from the

quarantine facilities that the following documentation is required before material can be exported:

* a valid import permit for the destination country

* a document clearly stating the phytosanitary treatment of the material to be given prior to export from the UK.

RU Accession Numbers

We are now assigning a Reading University accession number to each clone as we take it into our quarantine facility, in a similar way to the PI (Plant Introduction) accession numbers used by USDA quarantine stations such as Miami. Our accession number is in the form RU 890201, where RU stands for Reading University and the number is coded to give the year (*e.g.* 1**89**) and month (*e.g.* **02** - February) when the consignment arrived and a number to represent the individual clone within this consignment (*e.g.* **01**). We will be including these accession numbers with the documentation accompanying any budwood we export and we hope that receiving stations will keep these numbers in their records and, in turn, pass the numbers on as they distribute the budwood to other collections. We hope that this will have a number of advantages in keeping track of germplasm distribution and in preventing clones becoming mislabelled. For example, in the past, there are instances where the same clone name has been assigned to two or more different genotypes and other cases where a clone name has been corrupted so that two or more different clone names refer to the same genotype. This has led to much confusion and wasted resources in germplasm collections. If a quarantine accession number is used in conjunction with the clone name, it should be possible to trace the original donor of the material from the quarantine facility's records. Similarly, if a clone in the original genebank or quarantine facility is found to be mislabelled it should be possible to identify collections which received material from this source and alert them to the problem.

Post-Entry Quarantine

A working group at the Workshop on the Conservation, Characterization and Utilization of Cocoa Germplasm, Trinidad in 1992 discussed the importance of post-entry quarantine in the safe movement of cocoa germplasm. We are currently preparing some guidelines for countries receiving budwood from our quarantine facility. If you would be interested in contributing to these guidelines or would like immediate advice on suitable quarantine procedures, please contact us at the address above.

International Cocoa Germplasm Database

The printed version of the BCCCA/University of Reading International Cocoa Germplasm Database (ICGD) is ready for distribution and has been sent out to cocoa research stations. This has been produced to complement the computerised version and will be especially useful to those who do not have access to a suitable computer.

We are preparing to release a new version of the computerised database early in 1994. Please send us a list of the accessions you hold in your germplasm collection and any information on the characteristics or performance of these accessions that you would like to be included in the database. If you would like a copy of the database, please send details of your computer such as disk drive size and type to:

Dr. Michelle End,
ICGD Project,
School of Plant Sciences,
Plant Science Laboratories,
University of Reading,
Whiteknights, PO Box 221,
Reading RG6 2AS.

References

Flynn, W.P., Glicenstein, L.J. and Fritz, P.J. (1990). *Theobroma cacao* L.: an axillary bud *in vitro* propagation procedure. Plant Cell, Tissue and Organ Culture, 20: 111-117.

Hughes, J. d'A. and Ollenu, L.A. (1993). The virobacterial agglutination test as a rapid means of detecting Cocoa Swollen Shoot Virus. Annals of Applied Biology, 122: 299-310.

Sagemann, W., Lesemann, D.E., Paul, H.L., Adomako, D. and Owusu, G.K. (1985). Detection and comparison of some Ghanaian isolates of cacao swollen shoot virus (CSSV) by enzyme-linked immunosorbent assay (ELISA) and immunoelectron microscopy (IEM) using an antiserum to CSSV strain 1A. Phytopathologische-Zeitschrift, 114: 79-89.

Description of clones held in the Cocoa Quarantine Collection at Reading University
October 1993

(Kew, UK)
Royal Botanic Gardens, Kew,
Richmond, Surry. TW9 3AB.
United Kingdom.

(USDA Miami)
Subtropical Horticultural Research
Station, 13601 Old Cutler Road,
Miami, Florida. 33158.

(CATIE)
Turrialba,
Apdo 25,
Costa Rica.

(CIRAD-CP)
CIRAD-CP,
BP 5035, 34032 Montpellier,
France.

(ICG,T.)
Cocoa Research Unit,
University of the West Indies,
St. Augustine, Trinidad & Tobago,
West Indies.

(CIRAD-GUF)
CIRAD-CP
Kourou
French Guyane.

(* = plant material available)

Clone Name.	Donor Genebank.	Date Budded.	Origin.	Reading Code.
* AMAZ 3-2	ICG,T	18-01-88		RU 880101
* AMAZ 5-2	ICG,T	18-01-88		RU 880103
* AMAZ 6	ICG,T	18-01-88		RU 880104
* AMAZ 6-3	ICG,T	18-01-88		RU 880105
* AMAZ 10-1	ICG,T	18-01-88	R. Amazon, Iquitos Island, Peru. Chalmers, 1973	RU 880106
* AMAZ 10-3	ICG,T	18-01-88		RU 880107
AMAZ 12	ICG,T	22-02-93		RU 930201
* AMAZ 12-4	ICG,T	10-12-87		RU 871209
* AMAZ 15	ICG,T	18-01-88		RU 880109
AMAZ 15-15	Kew, UK	24-07-92		RU 850001
BORNE 7 A2	CIRAD-GUF	21-09-93		RU 930917
BORNE 7 A6	CIRAD-GUF	21-09-93		RU 930918
BORNE 7 B2	CIRAD-GUF	21-09-93	Borne 7, French Guyana. Lachenaud, 1990	RU 930919
BORNE 7 B3	CIRAD-GUF	21-09-93		RU 930920
BORNE 7 B4	CIRAD-GUF	21-09-93		RU 930921
BORNE 7 B5	CIRAD-GUF	21-09-93		RU 930922
B 121	ICG,T	19-07-93		RU 930701
B 131	ICG,T	08-03-93	Hacienda Bolivar, Ecuador. Pound, 1937	RU 930301
B 137	ICG,T	08-03-93		RU 930302
* BE 2	CATIE	13-07-88		RU 880701
* BE 3	Kew, UK	06-07-88	Belem, Brazil. Vello, 1965	RU 850002
* BE 10	Kew, UK	06-07-88		RU 850003
* CC 11	Kew, UK	10-05-88	Cacao Centre, Costa Rica.	RU 850004
* CC 38	Kew, UK	30-01-90	UF 676 x MATINA, Costa Rica.	RU 850005
* COCA 3370-5	ICG,T	06-09-88	R. Coca, Ecuador. Chalmers, 1973	RU 880901
CRUZ 7-11	ICG,T	12-01-93	open pollinated seedling from C. Sul, Brazil.	RU 930102
EET 19	ICG,T	10-09-93		RU 930901
EET 58	ICG,T	10-09-93		RU 930902
EET 59	ICG,T	19-07-93	Estacion Experimental Tropical, Ecuador.	RU 930704
EET 95	CATIE	24-09-93		RU 930934
EET 103	CIRAD-GUF	21-09-93		RU 930923
EET 162	ICG,T	08-03-93		RU 930303
EET 272	Kew, UK	29-08-91		RU 850006
EET 400	CIRAD-GUF	21-09-93		RU 930924
* EQX 69	ICG,T	18-01-88	Selections from Ecuador.	RU 880110
* EQX 78	ICG,T	10-12-87		RU 871213

* EQX 3161	ICG,T	06-09-88		RU 880903
* EQX 3308-1	ICG,T	18-01-88		RU 880112
* EQX 3348-44	ICG,T	06-09-88		RU 880904
* EQX 3348-52	ICG,T	06-09-88	R. San Miguel, R. Coca, R. Payamino.	RU 880905
* EQX 3360-3	ICG,T	06-09-88	Ecuador. Chalmers, 1970	RU 880906
* EQX J5	ICG,T	21-02-88		RU 880201
* EQX O	ICG,T	06-09-88		RU 880902
EQX Z	ICG,T	21-02-88		RU 880203
* GU 123 C	CIRAD-CP	11-10-88		RU 881001
* GU 125 C	CIRAD-CP	11-10-88		RU 881002
* GU 133 C	CIRAD-CP	11-10-88		RU 881004
GU 136 H	CIRAD-CP	03-04-89		RU 890401
* GU 144 C	CIRAD-CP	11-10-88		RU 881005
* GU 147 H	CIRAD-CP	03-04-89		RU 890402
* GU 154 C	CIRAD-CP	11-10-88		RU 881006
GU 168 H	CIRAD-CP	03-04-88		RU 890403
* GU 171 C	CIRAD-CP	11-10-88	R. Camopi, French Guyana. Sallee, 1987	RU 881009
* GU 183 H	CIRAD-CP	03-04-89		RU 890404
* GU 207 H	CIRAD-CP	03-04-89		RU 890405
* GU 221 C	CIRAD-CP	11-10-88		RU 881014
* GU 221 H	CIRAD-CP	03-04-89		RU 890406
* GU 239 H	CIRAD-CP	03-04-89		RU 890407
GU 249 H	CIRAD-CP	03-04-89		RU 890408
* GU 259 C	CIRAD-CP	11-10-89		RU 881017
* GU 290 H	CIRAD-CP	03-04-89		RU 890409
* GU 296 H	CIRAD-CP	03-04-89		RU 890410
* GU 341 H	CIRAD-CP	03-04-89		RU 890411
ICS 7	ICG,T	10-09-93		RU 930905
* ICS 16	ICG,T	21-02-88	Trinitario	RU 880204
* ICS 27	ICG,T	06-09-88		RU 880907
* ICS 43	ICG,T	06-09-88	Nic. Criollo	RU 880908
ICS 44	CATIE	24-09-93	Imperial College Selections, Trinidad.	RU 930936
* ICS 47	ICG,T	06-09-88	Criollo	RU 880909
* ICS 48	ICG,T	06-09-88		RU 880910
ICS 70	ICG,T	12-01-93		RU 930108
* ICS 100	ICG,T	21-02-88	Nic. Criollo	RU 880205
* IMC 11	Kew, UK	10-07-89		RU 850008
* IMC 14	Kew, UK	10-07-89		RU 850009
* IMC 20	Kew, UK	06-07-88		RU 850010
* IMC 54	Kew, UK	30-01-90	Iquitos Mixed Calabacillo. Peru. Pound, 1937	RU 850011
IMC 61	Kew, UK	24-07-92		RU 850012
IMC 67	Kew, UK	31-07-92		RU 850013
* IMC 83	Kew, UK	30-01-90		RU 850015
* IMC 96	Kew, UK	26-10-90		RU 850016
JA 119	ICG,T	08-01-93	Hacienda Javilla, Ecuador.	RU 930305
KER 2-E	CIRAD-GUF	21-09-93		RU 930925
KER 3	CIRAD-GUF	21-09-93		RU 930926
KER 5	CIRAD-GUF	21-09-93	R. Oyapock, French Guyana. Lacenaud, 1990	RU 930927
KER 6	CIRAD-GUF	21-09-93		RU 930928
KER 7	CIRAD-GUF	21-09-93		RU 930329
KER 9	CIRAD-GUF	21-09-93		RU 930930
* LAF 1	Kew, UK	10-05-88	Costa Rica.	RU 850017
* LCT EEN 28/S1	ICG,T	22-10-87		RU 871009
* LCT EEN 37 A	USDA Miami	27-09-88		RU 880912
* LCT EEN 37 F	USDA Miami	27-09-88		RU 880917
* LCT EEN 37 G	USDA Miami	27-09-88		RU 880918
* LCT EEN 37 I	USDA Miami	27-09-88		RU 880920
* LCT EEN 46	Kew, UK	10-07-89	R. Napo, R. Yacuambi, R. Villano, Ecuador.	RU 850019
LCT EEN 85	ICG,T	06-09-88	Allen, 1980 - 1985	RU 880911
* LCT EEN 127	ICG,T	22-10-87		RU 871011
* LCT EEN 162/1010	ICG,T	22-10-87		RU 871012

* LCT EEN 163 A	USDA Miami	27-09-88		RU 880942
* LCT EEN 163 D	USDA Miami	27-09-88		RU 880944
* LCT EEN 241 on 163 B	USDA Miami	27-09-88		RU 880949
LP 141	ICG,T	08-03-93	Hacienda La Paz, Ecuador. Pound.	RU 930306
LV 20	ICG,T	08-03-93		RU 930307
LX 28	ICG,T	08-03-93	Limoncillo Selections. Ecuador. Pound.	RU 930308
LX 47	ICG,T	08-03-93		RU 930309
M 8	ICG,T	10-09-93	Surinam, Myers, 1930	RU 930906
MA 12	CATIE	13-07-88	Manaus, Brazil. Vello, 1965	RU 880708
* MAN 15-2	ICG,T	09-12-87	Open-pollinated seedlings of MA 15	RU 871216
* MAN 15-60	ICG,T	09-12-87		RU 871217
MO 20	ICG,T	13-04-92	R. Morona, Peru. Pound, 1937	RU 890413
MO 81	Kew, UK	1985		RU 850020
* MOCORONGO	Kew, UK	30-01-90	Santarem, Para, Brazil.	RU 850021
* MXC 67	ICG,T	05-05-92	Chiapas, Mexico.	RU 880114
* NA 33		23-05-88		RU 880502
NA 682	ICG,T	15-11-88	R. Nanay, Peru. Pound, 1937	RU 881105
NA 776	ICG,T	15-11-88		RU 881110
NA 824	ICG,T	15-11-88		RU 881113
PLAYA ALTA 2	ICG,T	1989	Rio Orinoco delta Venezuela.	RU 890412
P 4 A	Kew, UK	24-07-92		RU 850022
P 4 B	Kew, UK	31-07-92		RU 850023
* P 7 B	Kew, UK	30-01-90		RU 850024
* P 14 B	Kew, UK	01-07-89	R. Nanay, Peru. Pound, 1942	RU 850025
P 16 A	Kew, UK	13-04-92		RU 850026
P 19 A	Kew, UK	24-07-92		RU 850027
P 19 B	Kew, UK	13-04-92		RU 850028
* P 10 (10P)	Kew, UK	30-01-90	Tabasco, Mexico.	RU 850029
* PA 7		23-05-88		RU 880501
* PA 56	Kew, UK	10-05-88		RU 850032
* PA 70	Kew, UK	29-08-91		RU 850033
* PA 88	Kew, UK	10-05-88	Parinari, Peru. Pound, 1937	RU 850034
* PA 107	Kew, UK	10-05-88		RU 850035
* PA 137	Kew, UK	26-10-90		RU 850036
* PA 175	Kew, UK	30-10-90		RU 850037
PA 300	Kew, UK	29-08-91		RU 850038
PINA	CIRAD-GUF	21-09-93	Pina, French Guyana. Lachenaud, 1990	RU 930931
* PUCALA 1	Kew, UK	29-08-91	Brazil.	RU 850039
R.B. 29	CATIE	13-07-88		RU 880713
R.B. 29	CATIE	24-09-93		RU 930939
* R.B. 33/3	Kew, UK	10-05-88		RU 850040
R.B. 39	CATIE	13-07-88		RU 880715
R.B. 41	CATIE	13-07-88	R. Branco, Brazil. Vello, 1965	RU 880716
R.B. 46	CATIE	13-07-88		RU 880718
R.B. 47	CATIE	13-07-88		RU 880719
R.B. 49	CATIE	13-07-88		RU 880720
R.B. 49	CATIE	24-09-93		RU 930940
RED 127	ICG,T	22-02-93	Red Amelonado from Amazon Valley.	RU 930206
RIM 189	CATIE	09-10-92	Chiapas, Mexico.	RU 921005

* SC 1	Kew, UK	30-01-90		RU 850041
SC 3	Kew, UK	13-04-92		RU 850042
* SC 4	Kew, UK	26-10-90	Seleccion Colombia.	RU 850043
* SC 9	Kew, UK	10-05-88		RU 850044
* SC 10	Kew, UK	01-07-89		RU 850045
SCA 6	ICG,T	26-03-91		RU 890414
SCA 11	ICG,T	19-07-93		RU 930714
SCA 19	Kew, UK	13-04-92	Scavina. Brazil. Pound, 1937	RU 850046
SCA 23	ICG,T	10-09-93		RU 930910
SCA 24	ICG,T	08-03-93		RU 930310
* SIAL 93	Kew, UK	29-08-91	Brazil.	RU 850047
SIAL 339	Kew, UK	24-07-92		RU 850048
SIC 5	Kew, UK	24-07-92	Brazil.	RU 850049
SJ 119	ICG,T	08-03-93	Hacienda San Juan, Ecuador.	RU 930311
SPA 4	ICG,T	22-02-93	Progeny of a fruit donated to Columbia by	RU 930208
SPA 9	ICG,T	31-07-92	Pound.	RU 890415
* SPEC 54-1	Kew, UK	10-05-88		RU 850050
* SPEC 160-9	Kew, UK	10-05-88	R. Papuri, R. Cauca, Colombia. 1952	RU 850051
SPEC 184-2	ICG,T	12-01-93		RU 930110
SPEC 194-75	ICG,T	12-01-93		RU 930112
TAP 3	ICG,T	10-09-93	R. Tapiche, Ecuador. Chalmers 1973	RU 930916
* TJ 1	Kew, UK	10-05-88	Criollo, Honduras.	RU 850052
* UF 221	Kew, UK	30-01-90		RU 850054
* UF 667	Kew, UK	10-07-89		RU 850055
* UF 676	Kew, UK	10-07-89	United Fruit Company selections from the	RU 850056
UF 677	ICG,T	22-02-93	Atlantic coast, Costa Rica.	RU 930213
* UF 705	Kew, UK	29-08-91		RU 850057