COCOA GROWERS' BULLETIN

Number 44 **August 1991**

Contents

Cadbury Ltd
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England

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EDITORIAL

In recent years many authors, including myself, have highlighted the need for improved cocoa planting material with higher yield potential, but more especially with greater tolerance, or even resistance, to the major pests and diseases of the cocoa tree. As cocoa producers and consumers rightly expect the use of pesticides to be gradually reduced and eventually phased out in future decades, then the need for cocoa planting material with improved resistance to pests and diseases must increase.

A very high percentage (an estimated 70%) of world cocoa production is grown by smallholders who can never be expected to carry out their own cocoa breeding programmes. They will always depend on other bodies for planting material and for funds to ensure that Governments carry out the necessary research. Therefore, international donors are to be expected to have to provide the small producer with suitable planting material. Little investment is currently being made by the well-endowed international donors in cocoa breeding programmes towards tolerance to any major cocoa pests or diseases. Indeed, international donors are currently saying that an on-going structural surplus indicates that less cocoa research is required. Such inactivity must be considered unacceptable in the light of the needs of an estimated 5-6 million smallholders who depend on cocoa for their livelihood and their very survival in many cases.

In order to promote thought and discussion, this issue of the Cocoa Growers' Bulletin, brings together articles relating to techniques of characterisation of primary genetic resources. Without viable techniques to assess disease resistance (and to a less extent pest resistance as well), we are unlikely to make any meaningful progress forward.

There have been minimal advances in cocoa breeding in the last decade. The development of new cocoa varieties will take many decades. Work must start now. It is to be hoped that major international donors will respond favourably by the allocation of sufficient funds for collection, maintenance and characterisation of cocoa germplasm as a first step to the development of planting material with improved resistance to pests and diseases.

ISSN 0045-7256 *R.A.LASS, Editor*

Cocoa Growers' Bulletin is published twice per annum by Cadbury Schweppes plc as an information service to cocoa growers, plantation staff, extension officers and research workers. It is distributed free with one copy being sent to each name on the current mailing list. Any correspondence including requests for names to be added to the mailing list, should be sent to R.A.Lass, Group Head, Agriculture and Environmental Affairs, Technical Development Department, V52, Cadbury Ltd., Bournville, Birmingham, B30 2LU, England. Articles are included on topics of interest to stimulate thought and debate. They do not necessarily represent the view of the Editor or of Cadbury Schweppes plc.

REVIEW OF PRODUCTION, CONSUMPTION, STOCKS AND PRICES

October 1990 - April 1991
S.A.Wakeling
Cadbury Limited

PRODUCTION

Our last review was based on Gill and Duffus Cocoa Market Report No. 338, dated September 1990. In their latest Report No. 340, dated May 1991, minor changes to the world crops from 1986/87-1989/90 have been made.

The forecast for the gross World crop of 1990/91 has been upwardly revised (from Report No. 338) by 25 TMT to 2452 TMT. The main changes from Report No. 338 were increases of 28, 27, 25, 20 and 10 TMT in Indonesia, Brazil, Ghana, Cote d'Ivoire and Ecuador respectively and decreases of 35, 25 and 15 TMT in Malaysia, Nigeria and Cameroon respectively.

CONSUMPTION

The Gill and Duffus Cocoa Market Report No. 340 reports that the consumption of cocoa has continued to show healthy growth rates despite the forecast fall in the Soviet grind as a consequence of the economic and political changes currently being experienced in the USSR.

One of the most dramatic changes in grindings has occurred in Brazil. Gill and Duffus expect the growth in grindings experienced during October 1990 to March 1991 to continue during the second half of the crop year 1990/91. They are forecasting that the 1990/91 Brazilian grind will reach 265 TMT, exceeding the previous highest crop year grind of 247 TMT in 1984/85. They believe that a combination of factors led to this expansion:

* high butter ratios leading to attractive prices for combined product sales
* internal bean prices have recently been at relatively low levels
* sufficient spare processing capacity

Gill and Duffus add the caveat that the longer term outlook for the Brazilian cocoa processing industry may not be so favourable. If some of the worst predictions on the effect of Witches' Broom on production are realised, factories may be facing a severe shortage of bean availability by the middle of the 1990's.

WORLD COCOA PRODUCTION DATA

Reproduced from Gill and Duffus Market Report No. 340
Dated May 1991
(all figures in thousand metric tonnes (TMT))

	1986/87	**1987/88**	**1988/89**	**1989/90**	**1990/91***
Cameroon	123	131	129	125	105
Cote d'Ivoire	620	674	849	710	760
Ghana	228	188	300	295	275
Nigeria	80	150	160	170	145
Togo	14	9	9	6	6
Others	38	40	33	33	29
Total Africa	**1103**	**1192**	**1480**	**1339**	**1320**
Brazil	369	402	343	366	396
Colombia	55	59	50	50	53
Ecuador	71	78	88	102	105
Mexico	38	44	41	43	44
Peru	10	12	11	11	11
Venezuela	12	12	10	14	14
Others	12	9	10	12	12
Total C. & S.America	**567**	**616**	**553**	**598**	**635**
Dominican Rep.	42	53	43	60	44
Others	12	11	8	10	10
Total West Indies	**54**	**64**	**51**	**70**	**54**
Indonesia	56	65	102	119	150
Malaysia	171	227	223	241	230
Papua New Guinea	31	33	48	41	41
Others	20	20	24	22	22
Total Asia & Oceania	**278**	**345**	**397**	**423**	**443**
WORLD TOTAL	**2002**	**2217**	**2481**	**2430**	**2452**
% change in production from previous season	2.2	10.7	11.9	-2.1	0.9

* **Forecast**

N.B Some figures may not add due to rounding

WORLD COCOA GRINDINGS DATA

Reproduced from Gill and Duffus Market Report No. 340
Dated May 1991
(all figures in thousand metric tonnes)

	1986/87	1987/88	1988/89	1989/90	1990/91*
Belgium	34	35	41	44	46
France	35	40	44	59	65
Germany (Fed. Rep.)	205	225	244	273	299
Italy	43	46	46	51	50
Netherlands	190	215	234	241	257
Spain	33	43	40	44	46
United Kingdom	94	100	112	120	131
Others	57	55	59	61	63
Total Western Europe	**691**	**759**	**820**	**893**	**957**
Eastern Europe	99	88	99	70	54
USSR	164	132	201	106	70
Total E. Europe & USSR	**263**	**220**	**300**	**176**	**124**
Cote d'Ivoire	100	105	119	110	110
Others	63	71	65	68	70
Total Africa	**163**	**176**	**184**	**178**	**180**
Brazil	232	236	201	235	265
Colombia	48	46	41	45	48
Ecuador	29	36	37	44	50
Mexico	36	40	40	43	44
United States	228	241	237	275	286
Others	59	60	58	63	64
Total America	**632**	**659**	**614**	**705**	**757**
Japan	36	39	40	40	42
Malaysia	36	40	47	72	95
Singapore	34	42	46	51	51
Others	52	58	69	80	82
Total Asia & Oceania	**158**	**179**	**202**	**243**	**270**
WORLD TOTAL	**1906**	**1994**	**2120**	**2195**	**2288**
% change in grindings from previous season	2.1	4.6	6.3	3.5	4.2

* **Forecast**

N.B For 1990/91 Germany includes East and West Germany
N.B Some figures may not add due to rounding

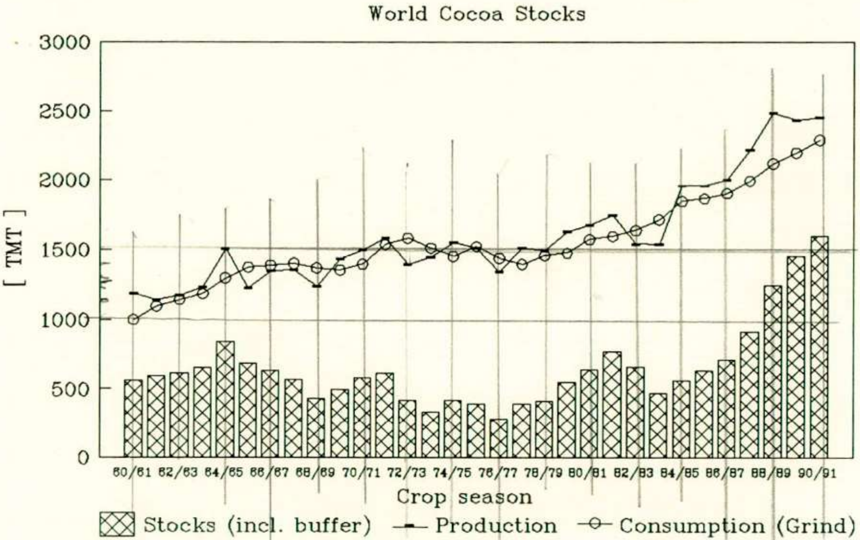
STOCKS

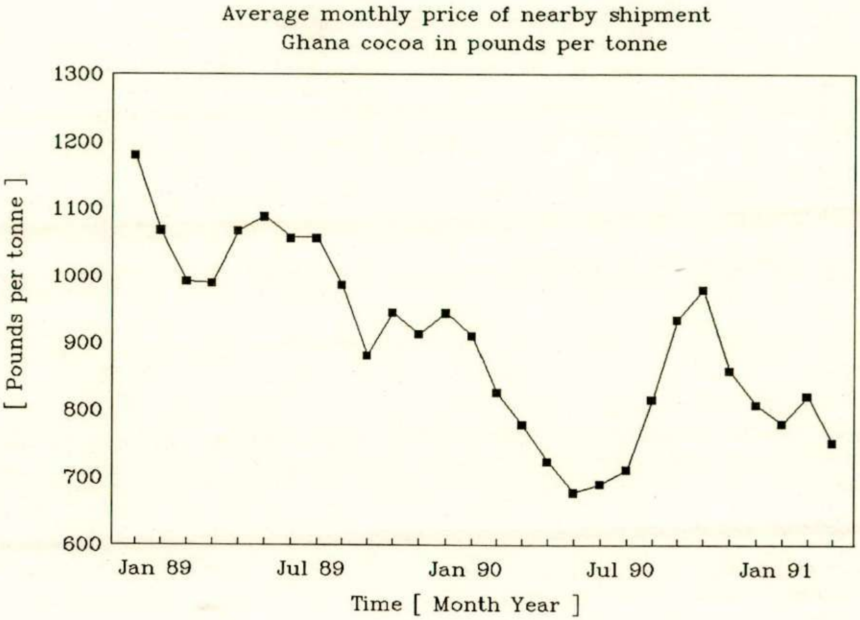
The continued production surpluses since 1984/85 have resulted in a large increase in world stocks both in absolute terms and as a proportion of world grind. The forecast for 1990/91 is a surplus of 139 TMT to add to the 1458 TMT of cocoa stocks at the end of the 1989/90 season.

WORLD COCOA STOCK DATA

Reproduced from Gill and Duffus Market Report No. 340
Dated May 1991
(all figures in thousand metric tonnes)

	1986/87	1987/88	1988/89	1989/90	1990/91
Closing stocks **(including buffer stock)**	711	912	1248	1458	1598
Stock/consumption ratio **(including buffer stock)**	0.37	0.46	0.59	0.66	0.70





PRICES

Gill and Duffus Report No. 340 reports that since January 1991 cocoa prices have fallen to life-of-contract lows on both the London and New York terminal markets. The main influence on price has been the inability of the USSR to allocate foreign exchange to finance cocoa purchases. This had a significant effect on people's perception of the current season's supply/demand situation. Further falls in the price of cocoa have occurred since March 1991. These falls were precipitated by reports of good rains in West Africa which were expected to improve the prospects of the mid-crops.

A HISTORY OF EARLY COCOA INTRODUCTIONS

G.A.R.Wood *

To write a history of cocoa introductions within the compass of an article for this journal one has to make clear just what this brief history is intended to cover: the starting point and the depth of detail. In this article I propose to start at the time when cocoa was first moved from Latin America and the Caribbean and then to mention the initial and subsequent major introductions to the other tropical countries where it is now grown.

THE EIGHTEENTH CENTURY

Before describing these movements, it is appropriate to discuss the changes that took place in Trinidad because Trinidad became an important source of planting materials during the 19th century.

Cocoa is said to have been introduced to Trinidad in 1525. This would have been Criollo cocoa and this type was grown until the 18th century when plantings were decimated by a "blast" in 1727. At one time the "blast" was thought to be a hurricane but it is now considered to have been a disease. Later, in 1757, Forastero material was introduced from Venezuela and this hybridised with the remaining Criollo, creating the Trinitario population which has become so well known. Originally this was due to the interest and skill of the planters who made selections and corresponded with similar groups of planters in Ceylon and Java.

By 1700 cocoa was being grown throughout Central America, in many of the islands in the Caribbean and in areas adjacent to the Andes in South America. Prior to this, cocoa had been introduced to south-east Asia. According to van Hall (1914) and Nosti (1953) cocoa was transported from Caracas eastwards to the Celebes in 1560 and Nosti states that cocoa was moved westwards from Acapulco to the Philippines in 1614, although 1670 is quoted by Wright (1907) and on the map in Nosti's book (Figure 1). Unfortunately these authors have not given any sources for these dates and so it is not possible to confirm the information. Whatever the correct dates - and 1560 seems remarkably early for a successful introduction over such a great distance - the type of cocoa moved must have been Criollo, the only type grown in the Philippines until quite recently and the type grown initially in Java. It seems probable that Java obtained its planting material from the Celebes or the Philippines. There is evidence that cocoa was taken from Amboina in the Moluccas to India in 1798 where it was planted in Tirunelveli District of Madras State; this again was a Criollo type (Ratnam, 1961).

The most significant movement of cocoa in the 18th century was the introduction of an Amelonado type from the Amazon basin of Brazil to Bahia. It is said that a Frenchman, Frederick Warneau, brought cocoa seeds from Para in 1746 and gave them to one Antonio

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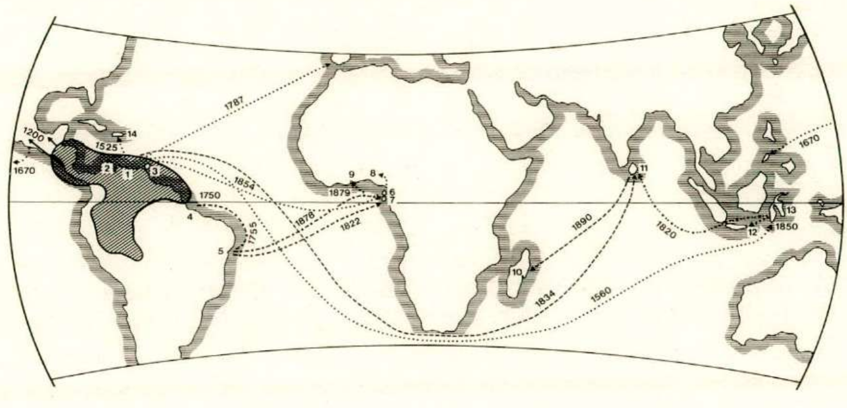
Dias Rubieso who planted them at Cubiculo de Almado on the banks of the Rio Pardo (Urquhart & Wood, 1954). However, many decades passed before Bahia became an important producing area.

FIGURE 1: ORIGIN AND DESTINATION OF PRINCIPAL MOVEMENTS OF COCOA 1200 TO 1879



Key:

Symbol	Nationality
································	Spanish
- - - - - - - - - - - - - - - -	British
————————————	Mayas
-·-·-·-·-·-·-·-·-·-·-·-·-·-·-·	Portuguese
··-··-··-··-··-··-··-··-··-··-··	Dutch

1	**Caracas**	**8**	**Nigeria**
2	**Maracaibo**	**9**	**Cote d'Ivoire**
3	**Trinidad**	**10**	**Madegascar**
4	**State of Para, Brazil**	**11**	**Ceylon (now Sri Lanka)**
5	**State of Bahia, Brazil**	**12**	**Java, Indonesia**
6	**Fernando Po (now Bioko)**	**13**	**Celebes, Indonesia**
7	**Sao Tome**	**14**	**Dominican Republic**

Source: **after Nosti (1953)**

Thus at the beginning of the 19th century cocoa production was confined to Central and South America and some of the Caribbean islands plus some plantings of local significance in the East Indies. The total world production in the first five years of the century averaged only 135,000 tonnes.

Great changes were soon to come due to much greater freedom of trade, reduction of taxes and hence increased demand for cocoa beans, followed by technical developments leading to a wider range of products being made from the cocoa bean.

THE SPREAD TO WEST AFRICA

There was considerable trade between Brazil and West Africa in the 19th century and not just between Brazil and Portuguese possessions in Africa - buildings in Lagos also bear witness to this traffic. Therefore the introduction of cocoa plants from Brazil to West Africa was bound to happen and 1822 is the date generally given for its introduction to Principe - a small volcanic island just off the coast of West Africa. From there cocoa moved to other islands in this chain - to San Thome in 1830 and to Fernando Po (now Bioko) in 1854 on the orders of Queen Isabel II of Spain (Nosti, 1953). A different origin is claimed by Cook (1982) who states that cocoa was introduced to Fernando Po by Spaniards in the 17th century from eastern Venezuela. He also states that the first introduction to Principe in 1822 was "probably from Fernando Po" and that the introduction from Brazil into Principe took place in 1830.

The subsequent history is fairly well known although the details may be vague and disputed. Cocoa cultivation in San Thome and Fernando Po depended on imported labour, workers being recruited in Angola for San Thome and in Nigeria for Fernando Po. Angola is generally unsuitable for cocoa, but Nigeria obviously is suitable and in 1874 Chief Squiss Ibaningo brought cocoa from Fernando Po to Bonny in the Niger delta (Ayorinde, 1966). Ayorinde states that the chief visited Madeira in 1871 and "there became interested in cocoa planting". However, cocoa has never been a commercial crop in Madeira, although it may have been grown in a botanical garden.

Better known is the introduction of cocoa from Fernando Po to the Gold Coast (now Ghana) by Tetteh Quashie in 1879. His was not the first introduction but it was certainly the most successful, partly because he had seen how cocoa could be grown while working in Fernando Po.

Earlier introductions had been made by various missionaries of the Basel Mission which had a station at Akropong (Wanner, 1962). The first of these efforts was in 1857 when some seeds were imported from Surinam. This failed as did subsequent introductions, possibly because the recalcitrant nature of cocoa seeds was not understood. Ill-health dogged these efforts in common with many other enterprises in West Africa in that era. However the Basel Mission can take credit for the fact that they played a part in the education and training of Tetteh Quashie.

Some of the seeds Tetteh Quashie introduced in 1879 were planted at Mampong-Akwapim in Ghana on the edge of the hills inland from Accra which proved to be the south-eastern limit of the cocoa area. His farm at Mampong succeeded and Akwapim farmers were quick to take up this new crop. The Governor, Sir Brandford Griffith, was also quick to realise the potential of the crop in the hands of local farmers and made further introductions of seed.

The type of cocoa was, of course, Amelonado and it was the basis of cocoa production throughout West Africa with the exception of Cameroon. When this country was a German colony a collection of planting material from South America and the West Indies was established in the botanic garden at Victoria. The result was a Trinitario population with many red-podded trees in West Cameroon, while in East Cameroon these Trinitario trees were mixed with Amelonado from Fernando Po. The different origin of this planting material may well account for the different physical characteristics (higher fat content and red coloured powder) of Cameroon cocoa as opposed to that of Ghana and Nigeria.

In Fernando Po a few plants of a Criollo variety were introduced in 1920 by one estate (Swarbrick, 1965) and this gave rise to a localised Trinitario population.

The major fresh introduction to West Africa was that of Amazon types. Over 100 pods were sent from Trinidad to Ghana in 1945 by Posnette (Toxopeus, 1985). This introduction covered a wide range of material arising from the work of the Imperial College of Tropical Agriculture and the Department of Agriculture in Trinidad, the latter having made numerous introductions from the Amazon basin in a search for trees resistant to witches' broom disease. Much of the cocoa planted in West Africa during the past 30 years arose from this introduction and in addition it provided the genetic material for breeding programmes. This is not the place to describe the Amazon material and its subsequent use but its importance cannot be over-emphasised. There have, of course, been subsequent introductions from Trinidad and the Cocoa Research Unit there continues to be the major centre for the study and evaluation of genetic material, most of it collected in the Amazon basin.

INTRODUCTIONS TO COUNTRIES IN ASIA AND THE PACIFIC

The early introductions to Celebes (Sulawesi, Indonesia) and the Philippines have been mentioned, as well as the movement from the Moluccas to India in 1798. While there is no firm record of the first introduction to Ceylon it seems probable that it was made at the same time, if not earlier, than the recorded introduction to Madras.

In the early part of the 19th century the plantation industry developed and botanic gardens were established; there arose a greater interest in introducing new crops and different, hopefully better, varieties of existing crops. Ceylon was one of the centres of such interest and there were several movements of cocoa in both directions between Ceylon and Trinidad during the 19th century. The first introduction from Trinidad took place in 1834-5 and the second in 1880. During the following 10-15 years Ceylon despatched cocoa plants to various countries. According to Wright (1907) cocoa plants were sent to Singapore and Fiji in 1880, to Samoa in 1883, to Queensland in 1886, to Bombay and Zanzibar in 1887 and to British Central Africa, presumably Tanzania, in 1893. Ceylon was also the source of material planted in Madagascar.

There is a record (Thong et al., in press) of cocoa in the garden of a Portuguese lady in Malacca in Peninsular Malaysia in 1778. It seems possible that the Dutch could have brought cocoa there earlier than this. A few experimental trees were reported to be thriving in Penang in 1802 (Thong et al., in press). The origin of the material in Malacca or Penang is unfortunately not known. Raffles encouraged efforts to plant cocoa in Singapore in 1823 which survived at least until 1866 despite the poor soil (Thong et al., in press).

MEANS OF TRANSPORT

While the type of cocoa involved in these introductions is often obscure and can only be inferred from recent knowledge of the types grown initially in the country concerned, the means of transport is even more obscure. We know that pods can be in transit for about two weeks without loss of viability; no doubt this has been known for a long time but many of the journeys mentioned would have taken at least as many months. During the later part of the 19th century Wardian cases were widely used for transporting plants. The Wardian case was invented by Dr Nathaniel Ward, an amateur botanist and medical doctor who practiced in the east end of London. The smoky atmosphere there was lethal to the ferns he wished to grow and the glass case was the answer. This formed the basis for the Victorian fern craze in England. Dr Ward saw its use for transporting plants overseas and sent plants from London to Sydney successfully in 1833 (Etter, 1971-3). Hinchley Hart (1911) describes in detail the Wardian case and how it was used for cocoa. These cases - a sort of mini-greenhouse - were typically 90 cm by 60 cm and at this size could hold 1200-1500 seeds or 100-150 year-old plants. Similar containers may have been in use in earlier times but there is no firm information about this.

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A HISTORICAL BACKGROUND OF THE COCOA RESEARCH UNIT, University of the West Indies, Republic of Trinidad and Tobago

J.A.Spence *

The Imperial College of Tropical Agriculture (ICTA) was established in 1921. Research in Cocoa at ICTA was initiated in 1930. The Colonial Office of the British Government was advised by the Lovat Committee to form a chain of Agricultural Research Stations to serve specific regions in the tropics. The emphasis was to be on long range programmes not usually undertaken by Department of Agriculture establishments. Prof. Cheesman, then Professor of Botany at ICTA prepared a proposal for biological research in cocoa which outlined priority needs for increase in yield and improvement of quality. Cocoa was then Trinidad's largest export crop. Overwhelming support was given to the programme by cocoa planters, government officials and cocoa traders in Trinidad. In 1929 the Colonial Office of the British Government approved the proposal and in 1930 a five-year scheme was launched. This has subsequently evolved into the Cocoa Research Unit (or CRU).

In 1929, contributions to administer the scheme were invited from other Governments and also from the Chocolate manufacturers in Great Britain. The following governments and companies promised financial assistance:

Government of Trinidad and Tobago
Government of Grenada
Government of Ceylon (Sri Lanka)
Government of Gold Coast (Ghana)
Government of Nigeria
Messrs Cadbury Bros Ltd
Messrs J S Fry & Sons Ltd
Messrs Rowntree & Co.

With an additional contribution from the Colonial Office the budget of £4655.00 per annum was utilized for the recruitment of three research officers, a Geneticist (F J Pound), a plant physiologist (E E Pyke) and a Soil Chemist (J A McDonald). The staff worked under the general guidance of Prof. Cheesman and Prof. Hardy. Pound's work culminated in the selection of 100 clones - Imperial College Selections (ICS 1-100) from among the cocoa populations of Trinidad. These were distributed world-wide. Meanwhile Pyke developed the first technique for rooting of cocoa cuttings which made possible the cultivation of clonal cocoa. McDonald carried out soil surveys and fertilizer trials and did some pioneering work on leaf analysis as a method of diagnosing the nutrient balance in the cocoa tree.

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In 1935, Pound joined the Trinidad Department of Agriculture as Cocoa Agronomist. One of the major contributors to Cocoa research was his exploration in Ecuador (1938) and the Amazon valley (1943) to collect wild cocoa types. The resulting germplasm collection is now in the International Cocoa Genebank, Trinidad which is managed by CRU. This material has subsequently played an invaluable part in all major breeding programmes for cocoa.

In the early 1940's, Prof. Cope carried out a selection programme in Grenada which resulted in the establishment of the GS clones.

After the war, a new and bigger scheme for cocoa research was financed jointly by the British Government (under the Colonial Development and Welfare Act) and the Cocoa, Chocolate and Confectionery Alliance Ltd, representing the manufacturers in the United Kingdom.

Recruitment of eleven Senior Research Staff was completed in 1950. Five major areas of work were carried out under the new scheme, namely:

1. Selection, breeding and propagation of cocoa
2. Soils and nutritional factors in relation to cocoa growing
3. Other environmental factors in relation to cocoa growing
4. Pests and diseases of cocoa
5. Preparation and quality of cocoa.

In the early 1950's a large scale development programme to encourage cocoa planting based on work done in the 1930's was launched by the Cocoa Board in Trinidad and Tobago. The main innovation in the scheme was to rehabilitate the Cocoa Industry using rooted cuttings of the ICS clones produced in large scale propagating facilities. Similar programmes were initiated in Grenada, St Vincent and St Lucia. A two year expedition (called the Anglo-Colombian collecting expedition) was mounted in Colombia to survey and collect new cocoa material.

In the mid-1950's, the emphasis in the plant improvement programme shifted from collection and selection to breeding. Using Amazon and ICS material as parents, SCA 6 and 12 crossed with ICS clones gave yields up to 3400 kg/ha at River Estate. The progenies also proved to be resistance to Witches' Broom Disease in Trinidad.

Major studies were made in nutritional aspects (deficiency symptoms), new propagation techniques, shade/nutrition interactions, manorial and cultural practices, screening technique for Witches' Broom resistance, wilt disease, physiological/ecological studies on the incidence of thrips, beetle and mealy bugs on cocoa, as well as on chemical changes and the significance of aeration and temperature during fermentation in relation to final chocolate flavour.

The Regional Research Centre (RRC) for the British Territories in the Caribbean came into being on 1st September 1955. The nucleus of the Centre had been formed from the staff of the three Research Schemes for bananas, cocoa and soils, which were wound up at that time. In the agreed five-year research programme effective from 1st September 1956 it is stated that "Research on Cocoa at RRC has two main practical objectives:

(i) the elaboration of techniques for removing factors that limit optimum yield

and

(ii) evolution of high yielding trees bearing beans of desirable flavour".

It was recognized that the research on cocoa within the RRC was not to be only confined to Caribbean problems but as far as local circumstances allow, was world-wide in its range.

In 1960, ICTA was incorporated into the University of the West Indies to become its Agricultural Faculty. The responsibility for the RRC had lain with the Council of Ministries of a number of West Indian Governments. During the 1960's, the West Indian Governments altered the priorities of research from export crops to food crops which resulted in a reduction of funds for research on cocoa. Due to the reduction in the contribution for research on cocoa at that time, the number of research staff was reduced and reorganized into the Cocoa Research Unit from 1st January 1963 with D B Murray as Head of the Unit.

During this period the American Cocoa Research Institute (ACRI) began to contribute to the budget of the Unit which resulted in the appointment of a Plant Breeder, W S Chalmers, for a co-operative programme with Ecuador. An important aspect of Chalmers' work was the collection of wild cocoa from the Amazon area of Ecuador to expand the germplasm collection in Trinidad.

The Unit struggled through a very critical decade (the 1970's) during which period the majority of the staff members left the institution, due to the uncertainty of long-term financial support from donors. The American Cocoa Research Institute withdrew its financial support to the Cocoa Research Unit and for some years the Unit was left with assured support only from the Governments of Trinidad and Tobago and Jamaica, and the UK Cocoa, Chocolate and Confectionery Alliance (CCCA now known as BCCCA). Fortunately most generous financial support from the EDF was made under the Lome Convention. The EDF approved financial assistance in 1981 when the Cocoa Research Unit received a grant of 950,000 EUA.

The EDF has supported the preservation and expansion of the germplasm bank (now called the International Cocoa Genebank, Trinidad or ICG,T), for collection of additional material and resiting existing material, and the dispatch of primary genetic material to various countries. There is no restriction on the international availability of any material in ICG,T once the material has passed through quarantine. There are presently some 20 scientists and postgraduate students working at the Cocoa Research Unit on a wide range of subjects associated with the collection, conservation, characterisation and cataloguing of primary germplasm. The present work programme is described in CRU Annual Report 1987-90 just published.* The work of the Unit over the period 1930-1980 is reviewed in 'Fifty Years of Cocoa Research in Trinidad and Tobago by A.F.Posnette'.*

Some opportunities for MSc or PhD studentships exist for students from ACP Cocoa Producers through EDF funding or from elsewhere for students of other nationalities. Those interested should contact the Head, CRU.*

The present work programme of CRU is targeted especially to work on genetic resources of cocoa and is best summarised in the 'CRU Mission Statement'. This has just been reviewed and agreed and is as follows:

1. To **conserve** on one site as a field genebank (to be known as ICG,T) all primary germplasm existing in Trinidad.

2. To **enlarge** existing collection of primary germplasm by:

   - The acquisition (through exchange if appropriate) of material previously collected in the Caribbean and in Latin America;

   - The conduct of expeditions to collect new material from the wild;

   - Maintenance of a cocoa quarantine facility in Barbados.

3. To **characterize** fully all the primary germplasm of ICG,T. This will include measurement of:

   - Heritable morphological characters (including leaf, flower, pod and bean characteristics);

   - Reproducible isozyme markers (using an appropriate number of enzyme systems);

   - Relevant economic information (shell percentage, fat content, fat quality and flavour characteristics) of dry beans;

   - Repeatable RAPD/PCR characteristics;

   - Levels of tolerance to **Phytophthora** and **Crinipellis** pathogens.

4. To **catalogue** all the data collected on the primary germplasm in ICG,T and incorporate it into the International Cocoa Germplasm Database (or ICGD).

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* Copies of these documents are available free of charge from the Head, CRU, University of West Indies, St Augustine, Trinidad, West Indies Telephone: 1.809.662.4996; Fax: 1.809.663.9686; Telex: 24250 UWIWG. Details of studentships are available from the same source

5. To **encourage** and **arrange** unrestricted international distribution through quarantine of primary germplasm from ICG,T on request. The material should be dispatched with as much information as possible on the above-mentioned characters. Further to develop and use tissue culture and/or micropropagation techniques for international distribution of germplasm under aseptic conditions.

6. To **produce** populations with enhanced levels of those genes which are of importance to plant breeders.

7. To **develop** and **adapt** appropriate scientific methodology to achieve the above mentioned objectives.

8. To **train** scientists from cocoa producing countries by offering a variety of research projects on cocoa to higher degree standard and to encourage the publication of the results of such work in the refereed scientific literature.

9. To **offer facilities** for visiting scientists to work on cocoa subjects of relevance to the world cocoa economy. Research may be on topics not covered by the 'Mission Statement' but should ideally utilize the wide range of primary germplasm at CRU and thus increase the knowledge of this material.

10. To **support** and **enhance** the cocoa research effort of Government of the Republic of Trinidad and Tobago (GORTT), in particular to identify the form of a 'model * cocoa plant' capable of significantly higher yields in an appropriate 'orchard' system and furthermore to **develop** a strategy for enhancing pollination to ensure the realisation of the full yield potential of the model plant.

11. To **collaborate** with institutions and universities world wide which have an interest in cocoa research.

The Cocoa Research Unit in Trinidad has played a crucial role in cocoa research throughout the world. Located near the centre of origin of cocoa, the Amazon Basin, the Unit has collected a range of genotypes which it has begun to analyse and plans to make available to cocoa producers, new material with greater disease, pest and drought resistance and also earlier bearing. Historically, the genetic base of cocoa grown in Africa and elsewhere has been very narrow, and with various pests and diseases a major problem in maintaining production. National research programmes have depended upon the introduction of new genetic material from Central and Southern America which has very often been screened, tested and developed in Trinidad.

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* In terms of plant size, shape, habit of branching, flowering and photosynthetic potential in relation to appropriate levels of moisture, temperature and light intensity

COCOA BREEDING REVISITED

J.M.Warren * and A.J.Kennedy **

INTRODUCTION

In 1987 a small group of geneticists published a review article in the Cocoa Growers' Bulletin, on the state of cocoa breeding around the world (Kennedy et al., 1987). They made a list of general recommendations as to the direction that cocoa breeding and germplasm utilisation should take in the future. Now four years on Dr.A.J.Kennedy, former geneticist at the Cocoa Research Unit, U.W.I, Trinidad and one of the original authors of the 1987 review, and Dr.J.M.Warren, the present geneticist with the CRU in Trinidad, look at the progress made during the intervening years. The future is again contemplated in the light of continuing loss of wild cocoa germplasm.

HISTORICAL BACKGROUND

Germplasm Collection and Conservation

Although obviously the history of cocoa breeding, germplasm collection and conservation has not changed during the past four years, it is necessary to recount some of the major areas of historical importance to compare and contrast them with the way forward.

The first germplasm collections of *Theobroma cacao* were assembled in the early part of this century (*see article earlier in this issue*). These collections were primarily breeders collections based on phenotypic selection of the 'best' locally cultivated trees. Subsequently many of these clonal selections were distributed around the world's cocoa research stations, where they can still be found today. This material appears in the pedigrees of many later breeding programmes. For fuller reviews of the history of cocoa breeding see Pursglove (1968) and Toxopeus (1969). In the 1920's and 1930's witches' broom disease became a serious problem in Trinidad. Dr F.J.Pound, then cocoa geneticist at the Imperial College of Tropical Agriculture, was unable to find sufficient disease resistance among his locally selected (Imperial College Selections or ICS) Trinitario populations. This situation prompted the first significant cocoa collecting expeditions. Pound's collecting trips of 1938 and 1943, therefore, had but a single aim; to search the cocoa populations of the upper Amazon regions of Peru and Ecuador for genotypes resistant to the fungus *Crinipellis perniciosa* (Pound, 1938).

Pound collected wild cocoa from along the Amazon and its tributaries in Peru and also selected material from commercial plantings in Ecuador. It is important to note that these

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upper Amazon accessions were 'selected' and do not represent the spread of natural diversity. Furthermore, the collecting in Peru was restricted almost exclusively to riverbanks since rivers were the only means of transportation. Unfortunately, the precise locations of these collections are now not known and so we cannot return to them to collect any more material.

Following quarantine in Barbados, Pound's collections were transferred to Trinidad and established at Marper Farm. This material has recently been re-established as young trees which are now maintained by the Cocoa Research Unit of the University of the West Indies in the International Cocoa Genebank, Trinidad (ICG,T).

The Chalmers collecting trips 1968 and 1972 (Chalmers, 1972) in Ecuador also collected material with similar economic objectives in mind. The joint Anglo-Colombian expedition (Baker, 1953) however, sampled germplasm using botanical criteria rather than economic ones. The International Cocoa Germplasm Database (End, 1991) sadly reveals that only 51 accessions from this collection remain extant. Plants from these collections are also represented in the ICG,T. These collections are shown in Figure I.

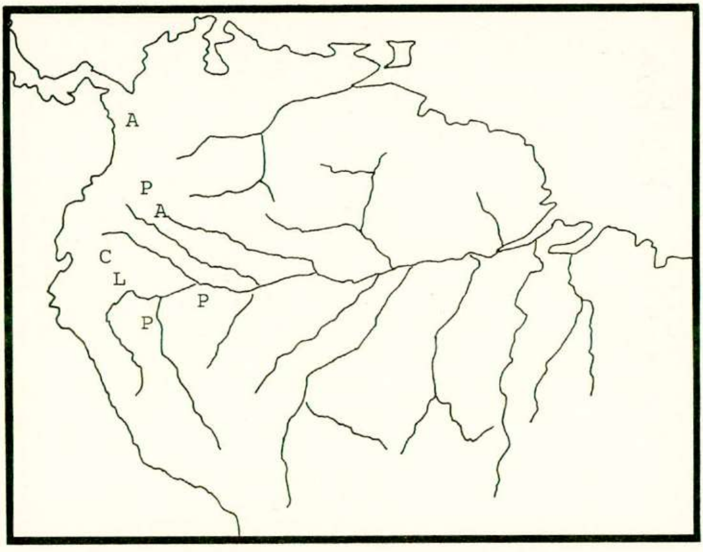

FIGURE 1: **A** = Anglo-Colombian Expedition
C = Chalmers Collections
L = LCTAP
P = Pound Collections

Crudely stated, the early breeders, when confronted with the problem of requiring genetic resistance to witches broom disease initially searched their own cultivated backyards. On drawing a blank there, they screened the then world genebank for cocoa namely, the wild rain forest populations, and the cultivated areas of Ecuador known to be heavily infested with witches' broom disease.

Arguably, not until the London Cocoa Trade Amazon Project (Allen and Lass, 1983) did anyone attempt to collect cocoa germplasm in a systematic and scientific manner. This expedition had the defined objective of broadening the range of genetic variation available to the breeders. The sampling strategy used was based upon Marshall and Brown (1975) and relied upon collecting 25-50 plants from many populations, rather than intensively sampling a few populations. The objective of this sampling technique was to collect all the alleles present in a population with a frequency of greater than 0.05. Rare alleles with a frequency below 0.05 were considered to be generally uninteresting to breeders. In reality rare alleles can be of great use to breeders, however, there are logistical problems involved in collecting and conserving them.

The IBPGR considers two main factors in determining priority for the collection of material. Firstly, what geographic areas are under-represented in existing collections, and secondly, what areas of variability are under-greatest threat (Williams, 1984). Materials of Criollo origin are considered to be a high priority for conservation because they fall into the second category. The Centro Agronomico Tropical de Investigacion y Ensenanza (CATIE) at Turrialba in Costa Rica holds a valuable collection of Criollo material which is currently being replicated at the ICG,T in Trinidad. Further collecting efforts for Criollo material would nevertheless be useful.

Breeding

With one or two exceptions notably the TSH programme in Trinidad (see Kennedy et al., 1987) crop improvement in cocoa has relied heavily upon the exploitation of heterosis rather than long term breeding programmes. Inter-population heterosis was first demonstrated in Ghana (Posnette, 1943) and later confirmed in Trinidad (Montserin et al., 1957). This extreme form of hybrid vigour is produced by crossing unrelated/geographically distinct populations of cocoa.

Hybrid seeds currently being released to farmers are often derived from crosses between upper Amazon Forastero types and locally selected clonal material. The lack of any relationship between the parents ensures that the desired heterosis is produced. The upper Amazon parent is commonly obtained from one of the IMC, NA, PA, or SCA populations collected by Pound in Peru. These plants are considered to provide desirable yield, vigour and disease resistant characters.

In Trinidad, the second locally selected parent is usually an ICS accession, and in Costa Rica, United Fruit Company (UF) clones are used. These local parents are believed to provide genes adapted to local conditions and pathotypes. Furthermore, the use of locally selected material as parents maintains regional flavour characteristics.

The segregation that results from crossing two highly heterozygous parent lines is of course massive although this can be masked by the initial heterotic vigour which appears to give uniform progeny. However, improved yield is the aim of the plant breeder rather than vegetative vigour. Commercial plantings of this hybrid cocoa demonstrate a high degree of genetic diversity and phenotypic variation. It is feasible that segregation of polygenic horizontal disease tolerance in this manner may enable virulent pathogens to evolve rapidly. This may occur, since in the F1 cocoa the genes for disease tolerance are diluted throughout the population. Pathogens may, therefore, evolve around each separate gene in a polygenic system in isolation. Inversely, quantitative traits, such as yield, cannot be improved by the mixing of desirable genes from several accessions when only a single generation is used in crop improvement. As a consequence of this simple crop improvement procedure, virtually all commercially grown cocoa can trace its ancestry back to a wild rain forest tree in only one or two generations. This is a fairly unusual situation for any crop species.

THE PRESENT

Germplasm Collections and Quarantine

The situation in the two germplasm collections recognized by the IBPGR (in Costa Rica and Trinidad) has not altered significantly since 1987. In Costa Rica, the collection is being re-established as young plants following problems with mis-identification of the existing accessions. The replanted collection is being arranged by geographic origin of the material. In Trinidad, all Pound's upper Amazon accessions have been regenerated from the original old trees held at the Marper Farm germplasm collection and on other private estates. The ICG,T is now located solely at the La Reunion site and now contains about 2000 accessions. Material continues to move into Trinidad, via quarantine in Barbados, from small collections in Central and South America.

The free flow of cocoa germplasm around the world is still restricted by the limited number of quarantine stations. The US Department of Agriculture centre at Miami remains the major effective recognized service. The BCCCA/Reading University Quarantine Station now also provides an alternative temperate quarantine facility. There are also plans to expand the small facility maintained by IRCC at Montpellier.

Questions should perhaps be raised about the expression of disease symptoms in pot grown cocoa in quarantine. Little is known at present about the expression of cocoa diseases under these conditions. Immunological tests may be required to determine whether non-virulent viruses are present in quarantined material. Clearly there is a case for further research to be carried out on the expression of disease symptoms in non-stressed young cocoa of various genotypes. There is also a need for clearly defined and internationally agreed upon guidelines regarding the operations of plant quarantine stations. At present there is no obligation upon stations to notify clients of suspected problems should any arise.

The Barbados quarantine station of the CRU in Trinidad is now operating efficiently following problems with water stressing although space is limited.

Biotechnology

Since 1987 there have been no major breakthroughs to report in cocoa biotechnology. The technical problems involved in regenerating cocoa plants from callus tissue remain unsolved. Thus the 'genetic engineering revolution' has not yet started for cocoa.

The development of successful micropropagation techniques is still expected to have a major impact on the movement of cocoa germplasm and management of genebanks, as it has in other crops. Given the current interest and research activity this breakthrough must surely be only a matter of time.

THE FUTURE

Germplasm Collecting and Collections

The future strategy of germplasm collection and subsequent management is influenced by the fact that neither pathogens or agronomic practices stand still. It is impossible to predict what new disease might be just around the corner or what cultivar will best suit future management practices. For these reasons, germplasm collections must never reject material simply because it is of no current economic interest. After all, the reject made today may be the wonder cultivar for high density planting which is needed tomorrow.

Genes which provide resistance to diseases which have not yet evolved onto cocoa will be present in natural populations at very low frequencies. This situation is analogous to the frequency of metal tolerance genes in populations growing in non-toxic sites, or herbicide tolerance genes in weeds before the application of herbicides. Furthermore, if disease resistance is polygenic then the frequency of individuals within a population which are resistant will be very low indeed.

The frequency of individuals in a population homozygous at five loci with individual allele frequency of 0.05 is:

$$f = (0.05)^{2x5} \qquad \text{approximately 1 in } 10^{13}$$

After Hallauer (1985)

As we have seen, the wild populations of *T.cacao* have been used by breeders in the past as a germplasm collection. These wild populations may or may not contain such desirable rare genotypes. The continued loss of cocoa germplasm from the wild and the simple economics involved in collecting, means that it now makes sense for breeders to obtain desirable genes from the two internationally recognized germplasm collections.

Sadly, although these collections contain a great diversity of material, much of it was either selected by plant breeders from the wild or represents the best of the locally cultivated material. Thus these collections cannot be said to contain the full range of natural diversity that may be required in the future. This situation will be confronted by both the proposed BCCCA/INIAA planned collecting programmes in Peru, by the CRU's proposed collecting trips in Trinidad and neighbouring islands and by other collecting programmes. These programmes will have the objective of broadening the genetic base of collected cocoa germplasm. This can only be achieved by using systematic and scientific criteria for selection of material collected, rather than economic ones.

Breeding

The breeding objectives proposed in 1987 still remain as desirable today. Unlike the germplasm collections which must hold material for unknown eventualities, breeders should have clearly defined goals for the future. As was pointed out in the 1987 article little attention has really been paid to yield improvement in cocoa. There is no point in a cultivar being highly disease resistant if it is low yielding. There is a need to identify the heritable components of yield and combine them via mass selection techniques. At some point the current reliance on heterosis for crop improvement must be abandoned in favour of longer term recurrent selection methods.

It is a sad reflection on the present state of cocoa breeding that there has been no significant progress to report in crop improvement since 1987. Although we have stressed a possible limitation of existing cocoa germplasm collections, there is more than adequate genetic variability already available to breeders to produce vast improvements in yield. It is the job of the local breeder to address this problem and utilise the considerable array of genetic resources which is already available.

The existence of genotype/environment interactions demands that breeding programmes be carried out locally to produce varieties adapted to local conditions. As was stressed in the 1987 article, evaluation of cultivars should be the responsibility of the local breeder not the manager of the germplasm collection. It is hoped, however, that a database catalogue of heritable variation held in the two germplasm collections will assist breeders in choosing which material to request from these centres. This information should be available in the not too distant future.

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ISOZYMES AND THE DESCRIPTION OF COCOA GERMPLASM IN TRINIDAD

G.Sirju-Charran * E.Johnson ** and J.M.Warren **

INTRODUCTION

The International Cocoa Genebank, Trinidad (ICG,T) maintained by the Cocoa Research Unit (CRU) of The University of the West Indies, St. Augustine is the most genetically variable cocoa germplasm collection in the world. Limited land space and the high cost of maintaining a collection of this size requires that there is no unnecessary duplication of the material.

A rapid and reliable method for the identification of accessions was required at the CRU to supplement the use of morphological descriptors. To this end, isozyme analyses by starch gel electrophoresis were initiated using peroxidase. The results obtained demonstrated that additional enzyme systems are needed to discriminate between accessions. The main objective of the current work is to discover enzyme systems suitable for characterization of the germplasm. Suitable enzyme systems are those which show variable isozyme banding patterns between accessions and which are stable over normal environmental conditions. Constitutive enzyme systems in flush leaves are being investigated. Ultimately it is intended that each accession will be described by a series of isozyme banding patterns which may be analogous to a fingerprint.

MORPHOLOGICAL DESCRIPTORS

The present method of describing accessions at the CRU is based on the measurement of leaf, flower, fruit and seed characteristics using the agreed IBPGR methodology (Anon, 1981). Although measurement of these morphological descriptors may be useful, this procedure is very time consuming and labour intensive. Furthermore these phenotypic descriptors may well be influenced by environmental variables. Flower, fruit and seed characteristics can only be recorded for sexually mature plants. Pod and seed data may need to be recorded anyway because they are of economic interest. There are thus a number of drawbacks to this methodology.

Isozyme analysis could be an effective tool for identification of accessions since analyses can be performed on tissue extracts from a plant at any stage of development. When the technique is perfected a large number of individuals can be rapidly evaluated. Isozyme descriptors are probably less likely to be influenced by the environment than are morphological characteristics.

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CLASSIFICATION AND ISOZYME ANALYSIS

Isozyme analyses by various electrophoretic techniques have been widely used to identify crop plant cultivars of barley and oats (Fedak, 1974; Almgard and Norman, 1970), sorghum (Shechter and De Wet, 1975), pear (Santamour and Denuth, 1980), grape (Walters et al., 1989), and cocoa accessions (Atkinson et al., 1986; Yidana et al., 1987; Lanaud, 1986).

At the Cocoa Research Unit, isozyme analysis using starch gel electrophoresis began with the study of the peroxidase system in leaf and bark tissue of cocoa (Yidana et al., 1987). Leaf and bark samples of the accession being studied were macerated to obtain tissue extracts. These crude extracts were absorbed onto filter paper wicks which were then placed in a starch gel. A direct electric current was applied to the gel for a specific period of time. This resulted in separation of the peroxidase isozymes according to their charge and mass. These isozymes were subsequently detected by placing slices of the gel into an appropriate staining solution. After a few hours, zones of isozyme activity appeared in the gel. The number of bands and the distance travelled was measured for each accession. These data were used to group accessions according to their banding patterns.

The number of peroxidase isoforms were greater in bark tissue than in leaf tissue of cocoa. Based on the banding patterns obtained in bark, six definitive bands were identified (Yidana et al., 1987). Using the observed variation of these definitive bands between accessions, three main reproducible patterns were obtained called Types ICS 1, IMC 67 and M 8 after the first accession in which they were identified.

Work on peroxidase in bark tissue of cocoa has continued at CRU for some two years with the analysis of about five hundred accessions. This yielded one additional reproducible isoperoxidase banding pattern. Some accessions were also observed to show seasonal variation implying peroxidase may be influenced by the environment. This observation, and the greater potential for detecting biochemically unmodified gene products in developing tissues, led to the decision to concentrate on flush leaf tissue rather than bark.

Currently the analyses of acid phosphatase (ACP), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), and shikimate dehydrogenase (SKDH) in flush leaf tissue are in progress on a routine. So far twelve reproducible banding patterns were obtained for MDH and eight for ACP. Using these two enzyme systems ninety-six combinations are mathematically, though not necessarily genetically, possible. Discrimination between most accessions within a particular peroxidase Type was achieved. A few of the ACP x MDH combinations however, contained more than one cocoa accession. Obviously, ninety-six groups cannot possibly characterize the two thousand plus accessions held in the genebank. Therefore additional enzyme systems are required.

Work on other enzyme systems is promising. PGI and IDH appear to be polymorphic and research is continuing. SKDH and LAP exhibit very little variation between cocoa accessions and so do not satisfy the criteria. Work is continuing on other constitutive enzyme systems in flush leaf tissue to find polymorphic systems which can be used to further characterize cocoa germplasm. Ideally only a few systems will be needed to characterize each of the two thousand plus accessions.

FUTURE

Another approach being developed at the CRU is to make use of enzyme systems which can be analysed on a minimum number of buffer resolution systems. In this way maximum benefits may be derived from the use of starch gel electrophoresis as a gel can yield several slices, each of which can be stained for a different enzyme system. This will speed up the work and decrease the cost of analysis of accessions.

In addition to identification of germplasm, the data obtained from electrophoretic isozyme analyses can perhaps be used for the assessment of the genetic variation in the populations present in ICG,T. This can be estimated by determination of allele frequencies from the isozyme data. Enzymatic markers have also been used to detect major genes contributing to quantitative traits. This has been done in tomato (Tanksley et al., 1982), and may be of value in the planning of cocoa breeding programs.

It has also been suggested that economically important, quantitative polygenic traits, such as yield (Tanksley et al., 1982; Frei et al., 1986) and disease resistance (Stavely and Hanson, 1967) may also be associated with isozyme markers. This is possible if the enzyme marker is linked to a major gene influencing these traits and so research is also in progress at the CRU to detect major genes contributing to traits of economic importance in cocoa.

Isozyme analyses should assist in documentation of ICG,T, whose objective is to conserve wild cocoa genotypes as a living genebank. The cost of maintaining such a collection can be greatly reduced if incoming material can be screened on arrival to evaluate its genetic importance. This should decrease duplication, redundancy and ensure conservation of genotypes rather than phenotypes.

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CHARACTERISATION OF COCOA CLONES USING DNA-BASED MARKERS

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INTRODUCTION

Concern over the genetic erosion of crop plants has led to an increase in plant exploration and in the establishment of numerous genebanks. Widespread deforestation particularly in South and Central America has resulted in the rapid loss of genetic variability for a range of tree species of the tropics. This factor has resulted in cocoa (*Theobroma cacao* L.) being designated as a priority crop for conservation (Anon, 1987). In order to provide a secure conservation base for the future, a sufficiently wide range of diversity is needed and two international "base collections" sited in Trinidad and Costa Rica have been designated. Plant exploration and collection is an important feature of the history of the crop (Allen and Lass, 1983) and many of the accessions collected are maintained by the Cocoa Research Unit, University of West Indies (Kennedy, 1984). The objective of such a living collection is to include with minimum redundancy, the genetic diversity of a crop species and its wild relatives (Brown and Clegg, 1983). This definition emphasises the need for a systematic evaluation and characterisation of the genetic resources present in a living collection.

The difficulty and expense of maintaining perennial tree crops in field genebanks, places great pressure on the evaluation procedure for such crops as cocoa. Traditionally, cocoa genetic resources have been characterised using a combination of morphological and agronomic traits. The effectiveness of using these to estimate genetic diversity has been questioned by several workers (Gottlieb, 1977; Brown, 1979). The long generation time of most perennial crops also means that many of the morphological descriptors can only be assessed at maturity. Furthermore, some collections have become too large and diffuse (Holden, 1984) with the result that utilisation is discouraged rather than assisted. Priority must therefore be given to the development of methods for the precise evaluation of variability in collections. The methods must be cost effective and the technology appropriate to the Institute responsible for managing the collection. In this article we describe our research on the development of molecular tools for the characterisation of cocoa genetic resources.

METHODS OF ANALYSING VARIABILITY

Morphological traits have the disadvantage of being influenced by environmental factors and this may not accurately estimate or represent genetic relationships. These factors have prompted the development of protein and DNA markers.

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Protein Markers

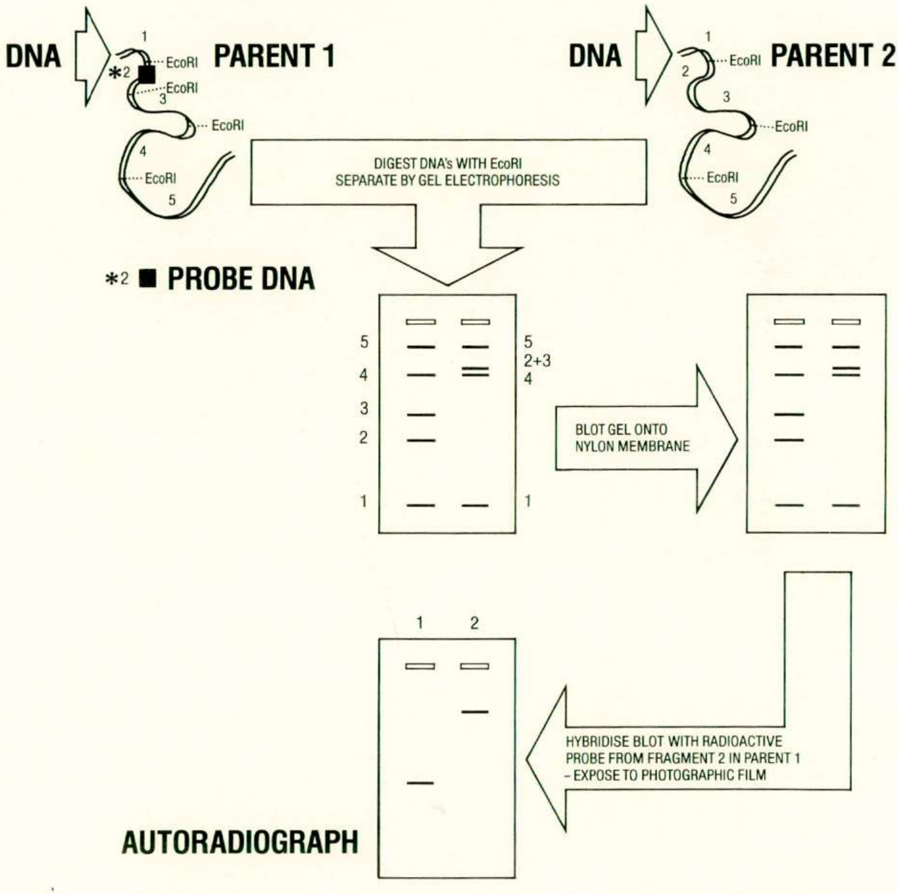
The most commonly used protein markers are isozymes. Isozymes are multiple molecular forms of enzymes that share a common substrate but differ in electrophoretic mobility (Markert and Moller, 1959). They are revealed when tissue extracts are subjected to electrophoresis in various types of gels and subsequently immersed in a solution containing enzyme-specific stains. Twenty-four enzyme systems have been examined in *Theobroma* (Atkinson et al., 1986; Yidana et al., 1987; Lanaud and Berthaud, 1984) and eight have been shown to be polymorphic. Although technically relatively easy to use, the low level of protein polymorphism detected is a limiting factor for fingerprinting cocoa. In addition, a number of different stains are required to identify polymorphism.

DNA Markers

Advances in molecular biology have resulted in new methods for the detection of polymorphism. Polymorphism in this case is revealed as differences in the lengths of homologous DNA fragments from different individuals. To detect these differences, the most frequently used technology relies on the use of a special class of enzymes called restriction enzymes. These enzymes have the ability to recognize and cleave DNA at target 'restriction sites'. These 'restriction sites' are locations at which exist specific sequences of the four bases, which when strung together, make the long DNA molecules which form the genome. A given restriction enzyme will therefore cleave a large fragment of DNA into smaller fragments. The size and number of fragments generated will reflect the distribution of restriction sites in the DNA and, after separating the fragments according to their length by electrophoresis, can produce a genetic fingerprint of the DNA of the organism under study. However, because the plant genome is so large, the number of fragments generated by restriction enzyme digestion is too great to resolve individual differences by electrophoretic techniques alone. This problem is overcome by exploiting the double-stranded nature of DNA (maintained by complementary base pairing) using a technique called DNA:DNA hybridisation. In this technique a small piece of cloned DNA from the plant under study (usually about $^1/_{10}$ - $^1/_5$ the size of the average restriction fragment length) is labelled with radioisotopes, made single-stranded, and used to 'probe' membrane filters on to which the total digested genomic DNA has been transferred. The probe molecule only binds tightly to the few sequences in the digest which have an exactly complementary DNA sequence. Because the probe is radioactive, the fragments to which it has bound can be visualised simply by exposing the hybridisation filter to photographic X-ray film. The differences detected between individuals are called Restriction Fragment Length Polymorphisms or RFLPs. The approach is illustrated diagrammatically in Figure 1.

DNA from several Amelonado trees has been isolated and digested with four restriction enzymes (BamHI, BglII, EcoRV and HindIII). The DNA was separated by electrophoresis and transferred to a membrane for subsequent hybridisation with radioactively labelled chloroplast specific probes. The polymorphism detected is illustrated in Figure 2 and indicates that the three cocoa genotypes differ in terms of their chloroplast DNA types. This is the first report of chloroplast DNA polymorphism in *Theobroma*. Although RFLPs can be used to characterise cocoa genetic resources, the technology is labour intensive requiring relatively large amounts of uncontaminated DNA which is difficult to obtain from cocoa. In addition, the isotopic labelling of a probe for use in RFLP studies can be a limiting factor particularly in developing countries. However, a DNA-based fingerprinting method requires just two steps: isolation of genomic DNA and determination of sequence variability.

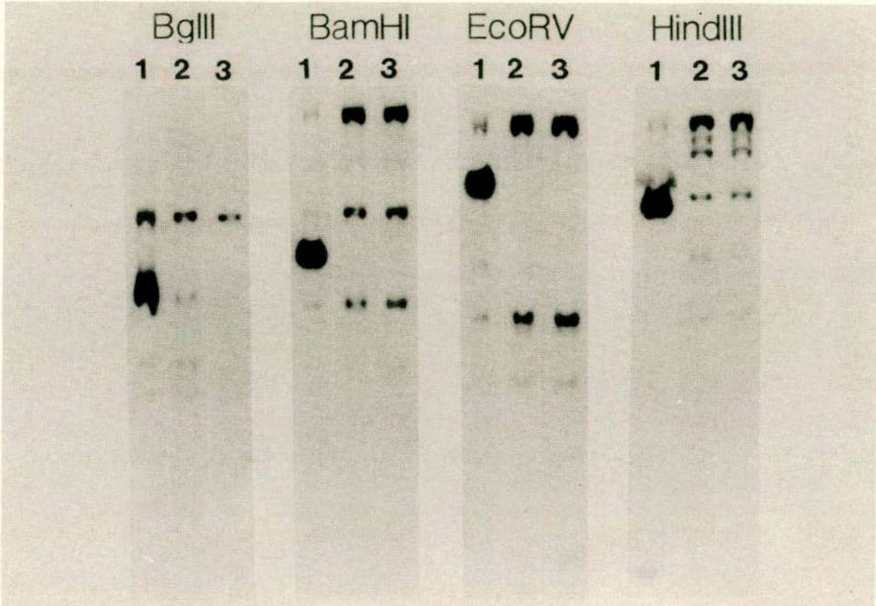
FIGURE 1: PROCEDURES FOR RFLP GENETIC FINGERPRINTING: DNA IS EXTRACTED AND CUT INTO FRAGMENTS BY RESTRICTION ENDONUCLEASES. THE DNA FRAGMENTS ARE SEPARATED ELECTROPHORETICALLY BY SIZE, TRANSFERRED TO A MEMBRANE AND HYBRIDISED TO RADIOACTIVE PROBES. HYBRIDISING FRAGMENTS ARE REVEALED BY AUTORADIOGRAPHY



POLYMERASE CHAIN REACTION (PCR)

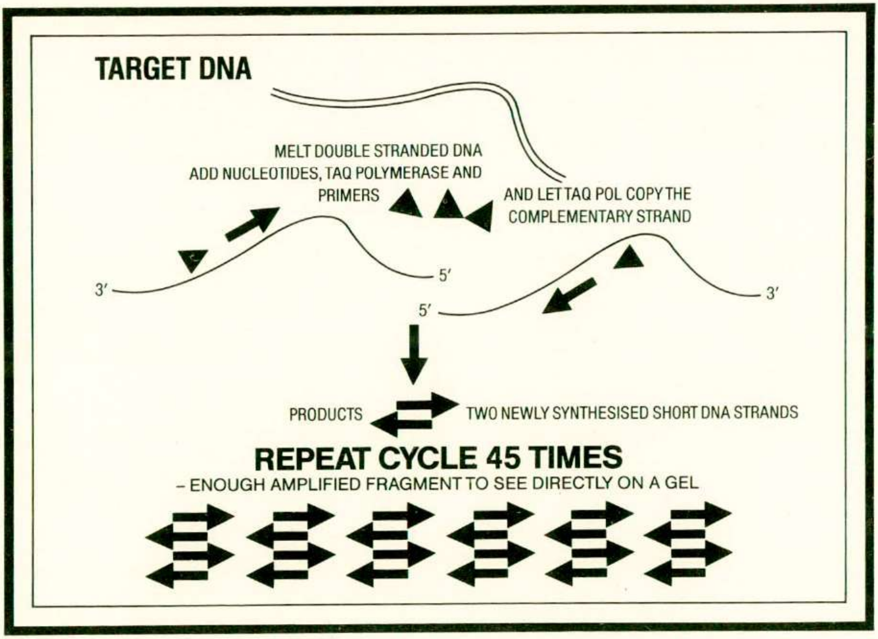
The PCR is based on a thermostable DNA polymerase enzyme (*taq*) which allows the selective amplification of a specific DNA fragment from a small amount of starting DNA. The technique depends on the ability of short, single-stranded DNA molecules called 'primers' to locate and bind to complementary sequences in the extremely long DNA molecule which comprises the plants genome. The *taq* polymerase catalyses the synthesis of a complementary DNA strand in one direction starting from the site where the primer has bound. When two primer binding sites are located on opposite strands of a DNA molecule within a given distance and in the right orientation (i.e. pointing together), repeating the PCR process through several cycles (which is possible because the *taq* is thermostable) results in an exponential amplification of the DNA between the two primer binding sites. The process is outlined in Figure 3.

FIGURE 2: DNA HYRBRIDISATION ANALYSIS OF THREE COCOA CLONES DIGESTED WITH FOUR RESTRICTION ENZYMES AND PROBED WITH A CHLOROPLAST SPECIFIC SEQUENCE



In the original form of PCR, it was necessary to know the sequences on either side of the target DNA before synthesizing complementary primers or oligonucleotides. However, recently it has been demonstrated (Williams et al., 1990; Wilde et al., 1991) that single short oligonucleotides of arbitrary sequence may be used to specifically amplify DNA fragments which can be visualised simply after electrophoresis by staining with ethidium bromide. The primers used have normally been only ten nucleotides long (10-mers). This procedure, known as Randomly Amplified Polymorphic DNA (RAPiD) analysis, has been successfully used in our laboratory to genetically fingerprint a range of *Theobroma* clones. The range of different phenotypes identified with a single primer for 13 *Theobroma* genotypes is shown in Figure 4. To date, our results demonstrate that the level of variability detected in *Theobroma* using RAPiDs is far greater than that observed with other polymorphism assays. Furthermore the RAPiD analysis is reproducible and allows discrimination both between, and within, *Theobroma* groups.

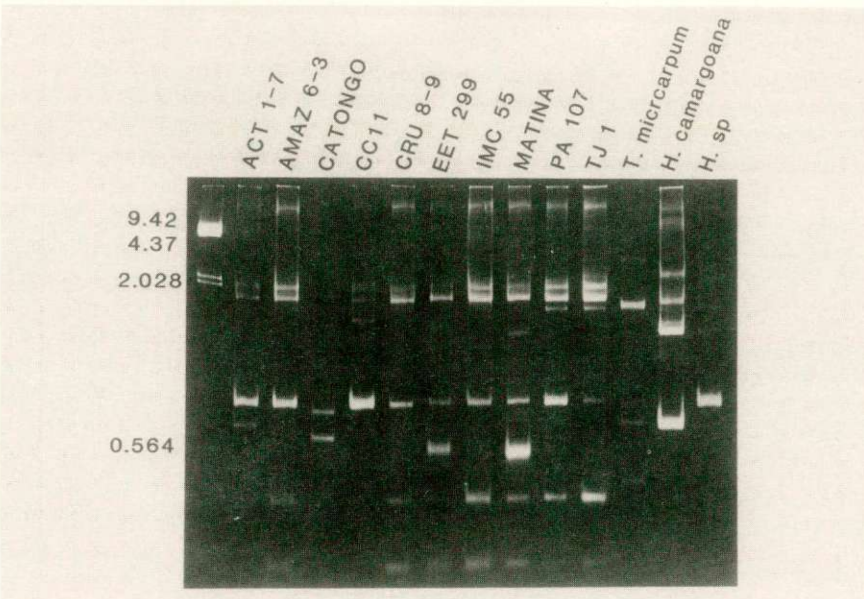
FIGURE 3: PCR-BASED TECHNIQUE FOR THE PRECISE AMPLIFICATION OF GENOMIC DNA



CONCLUSIONS AND FUTURE PLANS

Conventional RFLP analysis is labour intensive and requires relatively large quantities (5 ug) of uncontaminated DNA, which is difficult to obtain from cocoa (Couch and Fritz, 1990). PCR-based polymorphic assays are technically simple, easily automated and require only small amounts (100 ng) of DNA which can be extracted using mini-prep procedures from juvenile cocoa tissue. The methodology is not dependent on radio-isotopes and is therefore easily transferable to laboratories without facilities for radio-isotopes. In addition, the primers identified in our studies could represent a series of descriptors which would facilitate the characterisation and evaluation of cocoa genetic resources in field genebanks. The exploitation of RAPiDs would assist in the confirmation of accession identity, elimination of duplicates, allow the rationalisation of existing genebanks and permit future cocoa collecting expeditions to be targeted towards geographical areas possessing maximum levels of genetic diversity.

FIGURE 4: RAPiD ANALYSIS OF GENOMIC DNA USING A SINGLE SHORT OLIGONUCLEOTIDE PRIMER PRODUCING A GENETIC FINGERPRINT FOR 13 COCOA GENOTYPES



Presently, facilities are being established at the Cocoa Research Unit, University of West Indies, Trinidad for the evaluation of cocoa germplasm via the use of RAPiD markers. This important initiative has a crucial role to play in the future cost-effective characterisation and management of cocoa genetic resources.

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ACKNOWLEDGEMENTS

Research on the molecular characterisation of *Theobroma* clones has been financially sponsored by the Biscuit, Cake, Chocolate and Confectionery Alliance.

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Born in 1948 in United Kingdom, obtained BSc Honours in Biological Sciences at University of East Anglia, 1971, MSc in Applied Genetics, University of Birmingham, 1973, PhD from University of East Anglia working at John Innes Institute, 1975. Sugar cane breeder in Barbados until 1979. Fruit tree rootstock breeder at East Malling Research Station, Kent until 1982. Then joined Cocoa Research Unit, University of West Indies, Trinidad and has been instrumental in developing the present programme of genetic conservation in Trinidad building on the range of cocoa germplasm present on the island. Head of Unit 1984 to 1989. Now working as breeder at the West Indies Central Sugar Cane Breeding Station in Barbados and acting as Technical Adviser to the Cocoa Research Unit.

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Obtained BSc and MSc degrees from the University of Wales, Aberystwyth. PhD from the Genetics Department, University of Birmingham. Presently Head of Cell and Molecular Genetics Department, Scottish Crop Research Institute. Research interests include the study of genome organisation at the nucleic acid level and the exploitation of genetic markers for crop improvement.

DECEMBER 1990 cont'd ...

[Rubber (Hevea) as a permanent shade tree in cacao plantations] El caucho como sombrio permanente en plantaciones de cacao
RINCON S, O. - *Cacaotero Colombiano (Colombia)* v.12(37) p.33-41; Dec 1989.
The use of rubber (Hevea) as shade trees in cocoa plantations in Colombia is reviewed. The rubber trees (as a permanent shade) are sometimes interplanted with banana (as a temporary shade) and cocoa. Four combinations are shown, for instance: rubber at 12 x 3 m (278 trees/ha) combined with cocoa 3 x 3 m (1111 plants/ha) and banana 6 x 3 m (555 pl/ha). The various cultural practices for rubber growing, such as fertilizer measures are reviewed. The net return obtained with 1 ha cocoa under 200-250 rubber trees over 35 years is provided.

JANUARY 1991

Budwood deterioration and germplasm transfer in Theobroma cocoa
PURDY, L.H. Department of Plant Pathology, University of Florida, Gainesville, Florida, USA - *Turrialba (IICA)* v.39(4) p.435-440; Oct 1989.
Propagation failures at the cocoa quarantine facility in Florida, USA, prompted a study to determine the causes. Fungi were isolated from cocoa budsticks in Costa Rica (CR). Various treatments of budsticks were applied in CR and Florida, and buds were budded or grafted on rootstocks in Florida and CR. A low percentage of buds survived in Florida for 2 months, and only 22% survived in CR. The 5 most common fungi associated with cocoa budsticks were: *Botryodiplodia theobromae*, *Fusarium decemcellulare*, *Fusarium oxysporum*, *Pestalotiopsis spp.*, and *Phomopsis spp.* Certain fungi were found to be residents of the woody vascular cylinder of apparently healthy cocoa. Fungi within and on cocoa budsticks become active as a result of moisture loss by budsticks following their removal from donor trees. Certain fungi invaded the woody vascular cylinder within 2 days. Moisture loss from budsticks can be reduced or delayed by applying molten paraffin to the ends of budsticks and to the petiole stubs if they remain attached. Petioles should be cut 10-15 days before removing budsticks from donor trees. If patch or chip buds could not be used, the side veneer graft resulted in high survival of germplasm.

[Availability and processing of the by-products of cocoa] Consideracoes uties para o dimensionamento de projectos para aproveitamento dos subprodutos do cacau
PASSOS, F.J.V.and PASSOS, F.M.L.Centro de Pesquisas do Cacau, Comissao Executiva do Plano da Lavoura Cacaueira (CEPLAC), Itabuna, Bahia, Brazil - *Boletim Tecnico - Centro de Pesquisas do Cacau (Brazil)* no.167, 53 p. 1989.
Two by-products of the cocoa fruit, viz,pulp and sweet juice from the beans (mel de cacau), are produced in Bahia, Brazil. The juice is obtained by slight pressing of the beans. It contains only soluable acids, sugars and pectins. A good brandy is made from this juice. Refreshment drinks are made from the pulp, while the peel of the pods is used as cattle feed. Data on the availability of both by-products during the cropping season and about the equipment needed for processing purposes are given.

[Embryo culture from flat-shaped cacao seeds] Rescate in vitro de embriones provenientes de semilas aplanadas de cacao (Theobroma cacao)

PALMA, T. and VILLALOBOS, V.M. Instituto Tecnologico de Cartago, Cartago, Costa Rica - *Turrialba (IICA)* v.39(4) p.525-529; Oct 1989.

In Costa Rica a methodology was developed for embryo culture from flat-shaped cocoa seeds considered as haploids. The hybrids EET 62 x SCA 6 and Pound 7 x UF 613 were used. The hybrid embryos were cultivated in a Murashige-Skoog medium supplemented with thiamine-HCI 1 mg/1, pyridoxine-HCI 1 mg/1, nicotinic acid 1 mg/1, glycine 4 mg/1, inositol 200 mg/1, glutamine 200 mg/1, casein hydrolysate 200 mg/1, sucrose 4000 mg/1, and gel rite 1500 mg/1. The embryos were maintained at 27°c with a 16h photoperiod. The percentage of plantlets that developed varied from 16% to 87%, and plants obtained through this technique totalled 411. Of the developed plantlets, 2% were haploids.

Multivariate analysis and the classification of agricultural systems in a major tropical area of cocoa production (Barlovento, Venezuela)

BARRIOS, M., ARIAS, L. and SAN JOSE, J.J. Centro de Ecologia, Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela - *Turrialba (IICA)* v.39(4) p.477-482; Oct 1989.

Cocoa production systems of the Barlovento Valley, Venezuela, were classified according to multivariate statistical methods based on cluster analysis of cases and variables. A sample of 487 surveys were selected from a total of 850 farms according to the 3 main components of the production systems: producer's economic rationale, technology and natural environment. As a result of the cluster analysis, farms studied were divided into 5 groups, mainly influenced by factors unrelated to potential agricultural productivity. Thus, constraints such as high production costs, limited credit, restricted use of agricultural machinery, inadequate cultural practices and reduced human labour for agricultural activities establish priorities which have overriden consideration of biological production efficiency.

Cocoa nutrition and manuring in Malaysia

LING, A.H. Sapi Plantation Sdn Bhd, Sandakan, Sabah, Malaysia - *Planter (Malaysia)* v.66(771) p.302-312; Jun 1990.

Published reports on the nutritional requirements of cocoa with special reference to Malaysia are reviewed. The need for adequate and effective fertilizer applications for sustaining high yields were confirmed. The results of a number of fertilizer response experiments in Malaysia are reported, especially those involving response to NPK. Recommendations are given for effective fertilizer practice in young and mature cocoa.

Thermal characteristics and composition of fats from Theobroma species

CHAISERI, S., ARRUDA, D.H., DIMICK, P.S. and ENRIQUEZ, G.A. Department of Food Science, Pennsylvania State Univ., University Park, Pennsylvania, USA - *Turrialba (IICA)* v.39(4) p.468-472; Oct 1989.

The thermal behaviours and composition of fats from *Theobroma augustifolia*, *Theobroma bicolor* (from Costa Rica and Brazil), *Theobroma gileri*, *Theobroma grandiflora*and *Theobroma mammosa* were evaluated in comparison with cocoa butter. *Theobroma* fats, except for those from Costa Rican *T. bicolor*,exhibited lower solid fat contents (SFC) than those of cocoa butter at room temperature (20-25°C). This is the result of the high oleic and linoleic acid contents which made up more than 50% of their total fatty acid composition. Cocoa butter and Costa Rican *T. bicolor* contained 37.8% and 47.7% of oleic + linoleic acid, respectively. The melting behaviour of the Costa Rican *T. bicolor* fat was similar to that of cocoa butter, but its high SOO (S = stearic acid, O = oleic acid) content (22%) would be incompatible with cocoa butter. However, both Costa Rican and Brazilian *T. bicolor* fats contained high SOS. An SOS fraction could be used to improve the inferior quality cocoa butter.

World cocoa marketing systems, problems and priorities: an importer's perspective
SCHEU, J.J. - *Turrialba (IICA)* v.39(4) p.534-541; Oct 1989.
The price history for cocoa is discussed and the fundamental forces in price formation are identified. Various options in marketing cocoa and primary cocoa products are suggested. More attention must be paid to immediate post-harvest processing and quality control, farm and storage sanitation, individual contract performance and greater reliability of transportation. The need to diverisfy production and make it more price-reponsive, as well as improving accessibility of overseas markets, is explained. Methods for training marketing personnel are suggested, and the long-term prospects of cocoa production and consumption are explored.

Nutrient cycling in the cocoa ecosystem: rain and throughfall as nutrient sources for the soil and the cocoa tree
LETTE, J. DE O. and CALLE, R.R. Divisao de Geociencias, Centro de Pesqusias do Cacau, Itabuna, Bahia, Brazil - *Agriculture, Ecosystems & Environment (Netherlands)* v.32(1-2) p.143-154; Sep 1990.
Rainwater and throughfall water were evaluated as nutrient sources for a cocoa ecosystem in Bahia, Brazil. Rainwater was collected for 35 months in an open area. Throughfall was collected on shaded and unshaded cocoa plots. The shade trees were *Erythrina* and some species of *Ficus*. The concentrations of N, P, K, Ca and Mg, in both rainwater and throughfall varied with the presence or absence of shade trees in the cocoa plantation. The rainwater contributions of N, Ca, Mg, K, and P reached averages of 43, 21, 9, 9 and nearly 1 kg/ha/year, respectively. The averages of annual throughfall recycling to the soil in the unshaded and shaded plots, respectively, were 141 and 47 kg K/ha, 28.4 and 21 kg Ca/ha. The throughfall K and P appeared to be rapidly absorbed by the cocoa roots, whilst most of the Mg, Ca, and N were retained in the soil. The throughfall and leaf fall constituted the most important nutrient recycling processes in the cocoa ecosystem, which appeared to be self-sufficient in terms of its nutrient requirements.

Preconditioning of fresh cocoa beans prior to fermentation to improve quality: a commercial approach
SAID, M.B., JAYAWARDENA, M.P.G.S., SAMARAKODDY, R.J. and PERERA, W.T. Cocoa and Coconut Research Div., Malaysian Agricultural Research and Development Inst. (MARDI), Lower Perak, Malaysia - *Planter (Malaysia)* v.66(772) p.332-345; Jul 1990.
Studies were carried out on a commercial scale at Blenheim Estate in Lower Perak, Malaysia, to reduce acid content, and consequently improve the cocoa flavour. The method was developed based on the concept of pulp reduction of the wet beans by pre-conditioning prior to fermentation. Techniques adopted were pressing, air-blasting, and a short post-harvest storage followed by pressing and air-blasting prior to 5 days of fermentation. Fermentation was carried out in shallow boxes with one turning on the 3rd day. Although the normal practice of overnight storage of wet beans cause a reduction in pulp volume and content, it was found detrimental to the cocoa quality due to the accumulation of lactic acid. Pulp pre-conditioning through a combination of short pod storage and pressing followed by air-blasting gave a higher chocolate flavour strength than the beans fermented under normal estate practice. Besides an improvement in chocolate flavour, there was also a lower shell content and a better bean count.

[Cocoa/chocolate ratio of chocolate products exported from Brazil during the period 1970-1985] Relacao cacau/chocolate derivada dos productos chocolatados pelo Brasil, 1970-1985

ANDRADE, F.M. DE and BARROCO, H.E. Instituto Interamericano de Cooperacion para la Agricultura (IICA), San Jose, Costa Rica - *Turrialba (IICA)* v.39(4) p.542-548; Oct 1989.

The nature of Brazilian products exported as chocolate is discussed and the cocoa/chocolate ratio is defined. The methodology employed for the different types of chocolate aggregation is presented and is followed by a discussion of the nature and subsequent adoption of the conversion factors employed by the International Cocoa Organization. The data refer to the period 1970 to 1985. After a review of the literature dealing with chocolate and its classification (according to the Brazilian Commodities Nomenclature), only some of the products classified in this publication as chocolates were selected. With the subsequent application of the conversion factors a growing tendency of cocoa utilization in chocolate manufacturing was observed. This has led to the conclusion that Brazilian policies on export incentives should take into account the cocoa/chocolate ratio in export products with a view to establishing objectives, together with a means of assuring the improvement of rules and standards in line with chocolate production laws.

[An empirical model of the international cocoa market from the perspective of the Central American countries] Un modelo econometrico del mercado internacional de cacao desde la perspectiva de los paises centroamericanos

ROBINSON, H. Instituto Interamericano de Cooperacion para la Agricultura (IICA), San Jose, Costa Rica - *Turrialba (IICA)* v.39(4) p.511-518; Oct 1989.

An empirical model of the international cocoa market from the perspective of the Central American countries is described. The model provides a conceptual framework for the trade policy analysis and planning. The notion of "price taking behaviour" on the part of these countries was tested and verified using a simple econometric estimation, and resulted in the identification of adequate parameters and guidelines for designing an effective, successful production and export strategy for the Central American countries. The results clearly support the proposition that these countries face an infinitely elastic demand at the world equilibrium price level; as a result, the volume of their production (and export) does not affect it. The study revealed a statistically significant relation between the importing country's income and the price of cocoa. Some general guidelines for cocoa trade policy decisions derived from the study are discussed.

[Relation between the position of cocoa seed in the pod and the seed length, seedling height and stem diameter] Relacion entre la posicion de la semilla en frutos de cacao (Theobroma cacao), su longitud y el diametro y altura de las plantulas

PHILLIPS MORA, W. Programe de Mejoramiento de Cultivos Tropicales, Centro Agronomico Tropcial de Investigacion y Ensenanza (CATIE), Turrialba, Costa Rica - *Turrialba (IICA)* v.39(4) p.530-533; Oct 1989.

A study was conducted in Costa Rica to determine whether the seed position in cocoa fruits affected the seed length, seedling height and stem diameter. Seeds were extracted from the central and apical areas of fruits from cvs. SPA 9, IMC 67, EET 400 and UF 613. The seed length was determined and flat seeds were counted and discarded. Of the remaining seeds, 20 were sown per cv. The height of seedlings was determined at 23, 36, 57, 93 and 120 days, and the stem diameter at 1, 2 and 3 months. It was found that seeds originating from the fruit apex were shorter, and that flat seeds were few and only found in the apical areas of some fruits of cvs. EET 400 and SPA 9. The position of the seed within the fruit had no specific effect on the seedling stem diameter and seedling height.

[Cocoa seed storage] Conservacion de las semillas del cacao (Theobroma cacao)
SANCHEZ, J.E. and VELAZQUEZ, A. Programa Cacao, Campo Experimental Rosario Izapa, Tapachula, Chiapas, Mexico - *Turrialba* v.39(4) p.483-487; Oct 1989.
A study was undertaken in Chiapas, Mexico, to determine the capacity of polyethylene glycol(PEG) in preserving the viability of cocoa seeds, and to verify whether the presence of pod pieces, pulp juice or charcoal can be used to delay seed germination. The study was carried out in Petri dishes with 10 seeds of the RIM-117 clone per dish, using PEG (with molecular weights of 300, 1500, 3000, 4000 and 6000) in addition to tiram or chlorothalonil. Later, groups of 100 seeds in polythylene bags were used with pod pieces, pulp juice and charcoal. After 1 month storage at 28°C the percentage germination was evaluated by seeding on sterile soil. Charcoal was the best conservation material (72% germination) in groups of 100 seeds/bag; but the germination rate decreased in proportion to an increase in the number of seeds per bag. Storage in a aqueous solution in Petri dishes with PEG 3000 + thiram preserved 97.5% of seeds without husk. However, this method did not work with polythylene bags with 100 seeds. The incorporation of pod pieces and pulp juice did not preserve viability.

FEBRUARY 1991

[Estimation of the fertilizer requirement for cocoa in Litime (Togo)] Estimation des besoins en engrais des sols a vocation cacaoyere dans le Litime (Togo)
JADIN, P. and VAAST, P. Service d'Agronomie, Institut de Recherches du Cafe du Cacao (IRCC), Montpellier, France - *Cafe, Cacao, The (France)* v.34(3) p.179-188; Jul 1990.
As part of a project to regenerate cocoa plantations in Litime, Togo, by replanting with selected plant material, mineral fertilization to improve the growth and earliness of the young plants were envisaged. An investigation was carried out to estimate the soil chemical potential and to see if a standard fertilizer recommendation for all the village plantations in the region was possible. Soil analysis revealed that the soils were, on the whole, deficient in P, and, occasionally, N. The recommended standard formulae contain the following 3 fertilizers in variable proportions: triple superphosphate, KCI and NPK (15-15-15). The doses of each fertilizer were determined from histograms and the proportions may be calculated from the pH. Taking into account the current cocoa prices, mineral fertilization is still profitable in Togo.

Tenacity, biological activity and redistribution of copper fungicides on cocoa in controlled environments
RUDGARD, S.R., PETTITT, T.R. and HADLEY, P. Department of Horticulture, University of Reading, Reading, Berkshire, UK - *Crop Protection (UK)* v.9(4) p.281-288; Aug 1990.
The tenacity of 4 Cu-based fungicides exposed to simulated canopy drip was assessed on cocoa leaves and pods in relation to the concentration of fungicide deposit and the addition of Hyvis 150 adjuvant. The redistribution of localized deposits were determined over pod surfaces, and the release of Cu into surface water and runoff was assayed chemically for inhibition of spore germination of *Colletotrichum coccodes* and *Botrytis cinerea*. Cuprous oxide from Brazil eroded the most rapidly, the rate of loss increasing with surface dose. Hyvis reduced overall loss of Cu at low rates of application and less Cu was detected in runoff. Most of the Cu lost from deposits of all fungicides was eroded by the first 10 mm of rain equivalent. The release into water droplets on the surfaces of deposits was independent of deposit concentration in cupric hydroxide but not in cuprous oxide where values reached > 10 *u*g/ml indicating release of insoluable suspended Cu. Eroded deposits of cupric hydroxide released Cu in proportion to surface concentrations. A

stable fraction of deposits survived prolonged erosion but released little or no Cu into solution and was not biologically active.

MARCH 1991

Effects of VA mycorrhiza on the growth of cocoa seedlings under nursery conditions in Venezuela

CUENCA, G., HERRERA, R. and MENESES, E. Centro de Ecologia y Ciencias Ambientales, Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela - *Plant and Soil (Netherlands)* v.126(1) p.71-78; Aug 1990.

The effects of vesicular-arbuscular mycorrhiza (VAM) inoculation on the growth of cocoa seedlings (var. Ocumare 60) grown for 5 months in a nursery were studied in Venezuela. The effects of introduced VAM fungi *(Glomus occultum, Acaulospora appendicula, Glomus manihotis, Acaulospora morrowae and Scutellospora pellucida)* in soils treated with copper oxychloride or methyl bromide, were compared with the indigenous VAM and with their respective non-inoculated controls. Cocoa seedlings responded well to indigenous VAM fungi, which included *Scutellospora calospora* as the dominant species, inducing increases in height, dry weight and foliar uptake of P, Cu and Zn in relation to the sterile control. *Glomus occultum* and *A. appendicula* increased the height of cocoa seedlings but to an extent not yet statistically significant through the duration of the experiment. *Scutellospora pellucida* and *A. appendicula* doubled the P uptake of cocoa seedlings. The methyl bromide sterilization induced the lowest Cu and Zn uptake in the cocoa seedlings but this effect was ameliorated with VAM inoculation. Copper oxychloride treatment depressed growth to the same level as the sterile control although its residual effects did not kill VAM.

Ghana's cocoa pricing policy

BATEMAN, M.J., MEERAUS, A., NEWBERY, D.M., OKYERE, W.A. and O'MARA, G.T. - *Policy, Research, and External Affairs Working Papers - World Bank (USA)* no.429, 331 p.; Jul 1990.

The findings of a study for the Ghana cocoa Board on cocoa pricing policy are presented. Ghana's cocoa production declined because of policies that overvalued the domestic currency and heavily taxed cocoa exports. The formulation of cocoa policy appears not to have been linked with the world market. Yet cocoa is still the major source of foreign exchange earnings, supplying 50-60% of the total. This report consists of 4 parts, dealing with the analysis of cocoa pricing policy, cocoa tax and revenue alternatives, global perspectives on cocoa supply and demand, and the integration of policy applications. It is concluded that a variable rate tax on cocoa (above the critical level) that increases and decreases with the world price would distribute the price risk between cocoa farmers and the rest of society and stabilize cocoa farmers' real incomes.

Effect of spacing on yield in improved clones of Theobroma cocoa L.

MOOLEEDHAR, V. and LAUCKNER, F.B. Cocoa Research Section, Central Experimental Station, Centeno, Trinidad and Tobago - *Tropical Agriculture (Trinidad and Tobago)* v.67(4) p.376-378; Oct 1990.

A field trial was established in Trinidad in 1979 to investigate the effect of planting density on the yield of 3 improved cocoa clones. The 3 spacings tested were 3.6 x 3.6 m, 3.6 x 1.8 m and 1.8 x 1.8 m. From data collected over a 3-year period it was observed that all the clones performed significantly well under good conditions. Yields from the closest spacing were significantly better than the intermediate and traditional spacings. A variety/spacing interaction was detected with clone TSH 919 being better suited to the close spacing than the other 2 clones (TSH 730 and TSH 1188).

APRIL 1991

Studies on vegetative propagation of cocoa
HEDGE, S.G., SULEKERI, G.S. and HULAMANI, N.C. Department of Horticulture, University of Agricultural Sciences, Dharwad, Karnataka, India - *Indian Cocoa, Arecanut and Spices Journal (India)* v.13(3) p.93-95; Jan 1990.
Studies were carried out in Karnataka, India, during 1988 to enhance rooting of cocoa cuttings without mist. Cuttings were dipped for 60 s in a growth regulator solution of indole butyric acid (IBA) or naphthalene acetic acid (NAA) each at 2000 or 3000 ppm or their combinations. Cuttings were then planted in July (high humidity period). High humidity was maintained by a brick chamber or by a trench in the soil. Results showed that the trench maintained a higher relative humidity and gave 31% more rooting with vigorous root growth than the chamber. Moreover, IBA + NAA each at 2000 ppm gave the highest rooting of 67.78%, in the trench. The use of 2000 ppm of IBA and NAA each gave 53 and 33% rooting, respectively. The control (dipping in waer) gave the lowest rooting of 27.0% in the trench and 10.0% in the chamber.

Utilization of tropical foods: tropical oil-seeds
FAO, Rome, Italy - *Food and Nutrition Paper (FAO)* no.47(5), 98 p. 1989.
Various aspects of tropical oil-seeds used as traditional food crops in tropical areas are dealt with. This information includes nutritional value, household processing and distribution. The leguminous oil-seed crop presented in this book are: soya bean and groundnut, the non-leguminous oil-seed crops are: cotton seed, sunflower, safflower, nigerseed, sesame and bungu (*Ceratotheca sesamoides*). Also included in the book are: cruciferous oil seeds in India; economic aspects of edible oil production; cocoa; shea butter of shea nut; melons, pumpkins and gourds with edible seeds; and other edible oil-seeds. Tropical nuts from Central and Latin America (Brazil nut and cashew nut and cashew apple) are included.

Processing of cocoa beans at small scale level
GANESAN, V. and GEORGE, T.P. Department of Agricultural Engineering, College of Agriculture, Vellayani, Kerala, India - *Indian Cocoa, Arecanut and Spices Journal (India)* v.13(3) p.89-92; Jan 1990.
Processing of cocoa involves 2 steps: primary processing which includes fermentation and drying and secondary processing comprising roasting, kibbling and winnowing, alkalization, grinding and butter extraction. Appropriate technology and processing equipment were developed for small-scale processing of cocoa beans in Kerala, India. Equipment selected or designed and fabricated for both processing steps are indicated. An extraction unit for separation of butter from ground cocoa mass is described. The percentage of butter extracted at a pressure of 248.72 kg/cm^2 and a temperature of 70^{o}c. Palalable drinking chocolate and milk chocolate were made. The cost of production is given.

MAY 1991

Cocoa butter characteristics of Malaysian clonal cocoa
CHIN, A.H.G Food Technology Div., Malaysian Agricultural Research and Development Inst. (MARDI), Kuala Lumpur, Malaysia - *MARDI Research Bulletin (Malaysia)* v.17(1) p.37-44; Jun 1989.
In Malaysia 5 new cocoa clones (KKM 1, KKM 2, KKM 5, KKM 6 and KKM 7) were examined for their cocoa butter quality. Analyses of the chemical and physical properties, composition and cooling/melting behaviour of the fat showed that Malaysian cocoa butter from the new clonal material was similar to that from commercially planted material, despite inherent differences in composition among clones and between samples.

Therefore the tested cocoa butter was harder and had a higher melting point than the Ghanaian cocoa butter.

Towards the development of an integrated pest management system of Helopeltis in Malaysia

AZHAR ISMAIL Cocoa and Coconut Research Div., Malaysian Agricultural Research and Development Inst. (MARDI), Sungai Sumun, Malaysia - *MARDI Research Bulletin (Malaysia)* v.17(1) p.55-68; Jun 1989.

Various management approaches to control *Helopeltis* (especially *Helopeltis theobromae*) on cocoa in Malaysia are reviewed. As cocoa is a relatively new crop in Malaysia, management approaches are largely based on West-African experiences, and thus concentrated on chemicals. A list of recommended insecticides is included, as well as the application methods, interval and timing of the applications. Some cultural control methods such as the management of shade level, pruning and the planting of resistance varieties are discussed. Biological control by parasites and predators is not yet very common, but the use of ants in the development of integrated pest management (IPM) is worth considering. For the implementation of IPM, monitoring of the *Helopeltis* populations and the understanding of their ecology are important. The use of an early warning system may be one of the means to achieve IPM and thereby increase yield.

Tree crop based agroforesty in Nigeria: a checklist of crops intercropped with cocoa

RAO, M.R. and ROGER, J.H. International Council for Research in Agroforesty (ICRAF), Nairobi, Kenya - *Agroforestry Today (Kenya)* v.2(2) p.11-15; Apr 1990.

A survey of cocoa farmers in Bendel, Ogun, Ondo and Oyo, Nigeria carried out in 1985, showed that almost all the farmers intercropped other crops with cocoa. The intercrops included food crops such as plantain (92.3%), cocoyams (85.7%), cassava (51.3%), yam (41.3%), maize (38.9%), melon (31.4%), cowpea (28.6%) and pineapple (26.0%) and tree crops such as oil palm (71.5%), kola (67.3%), coffee (41.0%), coconut (7.0%) and citrus (7.2%). Other crops are ewe-iran (*Sarcophrynium brachystachs*) and ewe-gbodogi (*Megaphrynium macrostachyum*) (45.2%), conophore (42.2%), aligator pepper (*Aframomum melegueta*) (31.6%), and iyere (*Piper guineense*) (20.2%). As many as 6 or more other crops can be intercropped with cocoa at the same time.

Effect of intercropping on potential yield of cocoa in south western Nigeria

EGBE, N.E. and ADENIKINJU, S.A. Cocoa Research Inst. of Nigeria, Ibadan, Nigeria - *Cafe, Cacao, The (France)* v.34(4) p.281-284; Oct 1990.

The yields of experimental plantations in Nigeria, in which cocoa was interplanted with either kola, oil palm or *Terminalia ivorensis*, were examined. All the plots consisted of cv. Amazonian F3. Annual cocoa yields were recorded in plantations in which cocoa was grown alone and in plantations intercropped with one of the 3 plants cited above for a 10 year period, from the 10th post-planting year onwards. The cocoa-oil palm association was the most beneficial, with cocoa producing 67% more than in monoculture.

Smallholders cocoa production in Sabak Bernam: implications on economies of scale

MOHD. NOOR MAMAT and SHAABAN SHAHAR. Techno-Economic and Social Studies Div., Malaysian Agricultural Research and Development Inst. (MARDI), Kuala Lumpur, Malaysia - *MARDI Research Bulletin (Malaysia)* v.17(1) p.45-53; Jun 1989. In Selangor, Malaysia, the economies of scale practised by cocoa smallholders were studied. The most important contributory factor to cocoa production was land; the output supply elasticities of land, and fertilizer and labour inputs were 0.710, 0.053 and 0.133, respectively. The application of pesticides did not affect production. The sum of elasticities was about 0.9, but this did not imply decreasing returns to scale; it could be due to the exclusion of important variables, especially the age of the cocoa trees. The results indicated constant returns to scale in the smallholder cocoa production.

Rat spatial distribution pattern and its relation to pod damage in a cocoa-coconut planting system

KAMAL ADZHAM KAMARUDIN and LEE CHOON HUI. Central Research Lab Div., Malaysian Agricultural Research and Development Inst. (MARDI), Kuala Lumpur, Malaysia - *MARDI Research Bulletin (Malaysia)* v.17(1) p.75-82; Jun 1989.

In Malaysia, the spatial distribution pattern of rats in relation to cocoa pod damage in a cocoa-coconut intercrop was studied in 2 fields of 30 ha each for 20 months. The total numbers of rats caught and marked were 215 and 307 for each of the fields, respectively, which corresponded to the extent of pod damage. More were caught in tree tops than on the ground. Based on the rats caught on 4 or more occasions, an equal number of males and females showed a clumped distribution. A few animals exhibited a random movement, of which most were males. No rats showed hyperdispersion. The damaged pods were scattered and unevenly distributed in the 2 area. The K values (a measure of intensity of aggregation) for both fields were 1.16and 2.05, respectively, indicating clumped damage. The incidence of trees attacked also showed a low to moderate clumping with K values of 2.86 and 3.06. A slight correlation was observed between visitation pattern and pod damage.

Modelling agroforestry systems of cocoa (Theobroma cacao) with laural (Cordia alliodora) or poro (Erythrina poeppigiana) in Costa Rica. 5. Productivity indices, organic material models and sustainability over ten years

BEER, J., BONNEMANN, A., CHAVEZ, W., FASSBENDER, H.W., IMBACH, A.C. and MARTEL, I. Centro Agronomico Tropical de Investigacion y Ensenanza (CATIE), Turrialba, Costa Rica - *AgroforestrySystems (Netherlands)* v.12(3) p.229-249; 1990.

Predictive models were developed for laurel (Cordia alliodora) branch and cocoa branch or leaf biomass based on branch basal areas but the model of laurel leaf biomass, although significant, was of very low accuracy due to annual leaf fall. At the age of 10, shade tree stem biomass accounted for 80% of the total above-ground biomass. Between the ages of 6 and 10, the biomass increment of cocoa branches (3-4 t/ha/year) was similar to that of the shade tree stems. During the same period, the net primary productivity was 35 and 28 t/ha/year, for the *Erythrina poeppigiana* and laurel systems, respectively. Cocoa production under the shade trees laurel or *E. poeppigiana* was 1000 kg/ha/year (oven-dry; ages 6-10). During the same period, laurel timber production was 9 m^3/ha/year whilst the leguminous shade tree *E. poeppigiana* did not produce timber. Litter fall over the same 5 years, including crop and/or shade tree pruning residues, averaged 11 and 23 t/ha/year, respectively. The main difference was due to *E. poeppigiana* pruning residues (10 t/ha/year). Soil organic material reserves (0-45 cm) increased over 10 years from 198-240 t/ha in the *E. poeppigiana* plots and from 168-184 t/ha in the laurel plots.

JUNE 1991

[Cacao] Le cacaoyer

MOSSU, G. Institut de Researches de Cafe et du Cacao (IRCC), Paris, France - *Technicien d'Agriculture Tropicale (Nederlands)* Maisonneuve et Larose, Paris (France) no.14. 160 p. 1990.

This work is a concise and practical guide on growing cocoa, the handling of fresh pods, the production of dry beans and its processing into chocolate. The genus *Theobroma* comprises some 22 species, originating from the Amazon basin. The history, production centres, the various uses of cocoa, and a description of the 3 main classification groups, viz. Criollo, Forastero and Trinitario are given. Also included is the biology and ecology of the cocoa tree, propagation and breeding, various cultural practices, including the use of fertilizers, shade, pest control measures, harvesting and handling of fresh pods, drying methods, labour inputs expressed in man days/ha, quality standards and deficiencies of dry beans and the manufacturing of various chocolate compounds.

Observations on cocoa flushing in Ghana under different shade regimes, with particular reference to the distribution of natural light

ADOMAKO, D., FRIMPONG, E.B., AMPOFO, S.T. and BREW, K.M. Cocoa Research Inst., Tafo, Ghana - *Planter (Malaysia)* v.66(776) p.565-568, 571-579; Nov 1990

Unshaded cocoa trees growing on low wet ground, as well as shaded and unshaded cocoa trees growing on dry ground were studied in Ghana for the effects of sunlight availability and soil moisture on flushing. The studies covered the period of November 1982 to March 1987, which include the severe dry seasons of 1982/83 and 1983/84. Growth of cocoa seedlings and 1 year old cocoa plants at the gauze house and the nursery under 35% and 50% shade, respectively; was also studied. In 1983 and 1986 peak flushing occurred as usual in January when there had been little or no rain during the preceeding 10 and 4 weeks, respectively. Flushing was earlier, more frequent and more intense in unshaded trees than in shaded trees. There was no relationship between the flushing pattern and soil moisture content. Cocoa seedlings growing under medium shade flushed at regular (4 weekly) intervals without a seasonal peak. The 1 year old plants also flushed at regular intervals (of 4 to 5 weeks), each time at a few growing points; but as in the case of mature trees there was peak flushing involving all growing points during the drier part of the year (October-November and February-March). There was a highly significant correlation between sunshine hours and flushing.

[Integrated control of black pod and moniliasis of cacao in the humid tropical Atlantic zone of Costa Rica] Manejo integrado de la mazorca negra y la moniliasis del cacao en el tropico humedo bajo de Costa Rica

PORRAS, V.H., CRUZ C.A., and GALGINDO, J.J. Fundacion Hondurena de Investigacion, La Lima, Honduras - *Turrialba (IICA)* v.40(2) p.238-245; Apr 1990

The effect of sanitation (weekly removal of diseased pods), fungicide application and hand pollination on the incidence of black pod (*Phytophthora palmivora*), moniliasis (*Moniliophthora roreri*) and on cocoa yield was tested in the Atlantic zone of Costa Rica. Sanitation was economical and reduced the incidence of black pod from 10.3 to 6.7% and moniliasis from 70.1 to 44.8% and yield increased from 201 to 1101 kg/ha. The highest incidence of moniliasis was observed on pods <45 days old. With hand pollination, yield increased to 1534 kg/ha in plots with sanitation and to 2093 kg/ha in plots with sanitation and fungicide application. The use of fungicide brought no further benefits. Differences among cultivars in the incidence of black pod and moniliasis or in cocoa yield were significant.

JULY 1991

The status of cocoa (Theobroma cacao, Sterculiaceae) in the western hemisphere

HUNTER, J.R. Department of Botany, University of Wisconsin, Madison, Wisconsin, USA - *Economic Botany (USA)* v.44(4) p.425-439; Oct 1990.

The events that have led to the current status of selection, development, and breeding of cocoa are reviewed and alternative approaches are suggested. There are no long-term on-going programmes in any tropical country of the western hemisphere dedicated to the improvement of cocoa. While some effort is currently being made to obtain new acquisitions of cocoa cvs. exhibiting desirable characteristics and to maintain gene pools of these trees, there are few data from field trials to prove and substantiate these qualitites. In addition, there is growing concern regarding the disparities between predicted yields of cocoa trees through the use of hybrid seed and from actual production under field conditions. This has stimulated an awareness of the current inadequate understanding of the genetics of cocoa and the lack of comprehension as to which cultivars, under distinct ecological conditions, are precocious, resistant to disease, or heavy bearing, or indeed demonstrate those traits vital to the success of farming programmes adapted to today's market conditions.

An analysis of cocoa (Theobroma cacao L) yeild and its components
PALANIAPPAN, S. and SHUHAIMI SHAMSUDDIN FELDA Agricultural Services, Jerantut, Pahang, Malaysia - *Planter (Malaysia)* v.66(775) p.536-544; Oct 1990.
Two cocoa trials involving 25 progenies in trial 1 and 16 progenies in trial 2, were grown under inland conditions in Peninsula Malaysia. Results of a factorial analysis with respect to yield for both trials over 1979-1987, including yield components of bean weight, bean number and pod weight over 4 seasons between 1984-85 are presented. Pod production per tree and kg dry bean (kdb) production per ha showed significant differences among progenies for both trials. However, seasonal influence seemed more pronounced than progeny effect for pod production for both trials. The reverse was the case for kdb production per ha. Progeny x season interaction was non-significant for both trials and for both yield expressions. A factorial analysis of the pod weight, bean weight and bean number were carried out for 5 random progenies from trial 1, and 5 from trial 2 where UIT 1 was the common female parent. For the 1st trial, significant differences were noted for the bean weight and bean number while for the 2nd trial this was not the case. The bean weight was significantly influenced by the season but the pod weight was not. The bean and pod weight showed no significant interaction with season. Significant correlations for pod weight with bean weight and pod weight with bean number were obtained.

AUGUST 1991

Preliminary observations on the effects of bryophytes, lichens and other trunk infestations on flowering of cocoa
ADENIKINJU, S.A. and AFINFENWA, F.O. Cocoa Research Inst. of Nigeria, Ibadan, Nigeria - *Cafe, Cacao, The (France)* v.35(1) p.43-48; Jan 1991
The results of preliminary agronomic investigations into the effects of trunk infestation by bryophytes, lichens, mosses, bacteria, fungi, algae, ferns and small orchids on the flowering of cocoa cushions in Nigeria is reported. A plot of F_3 Amazon cacao was used for the study. Trunk infestation by epiphytes depressed flower production in 4 out of 12 months. The intensity of these effects, however, varied from month to month; this seasonability can be attributed to variations in rainfall and relative humidity.

Evaluation of systemic fungicides for control of witches' broom disease of cocoa in Trinidad
LAKER, H.A. Cocoa Research Unit, University of the West Indies, St Augustine, Trinidad and Tobago - *Tropical Agriculture (Trinidad and Tobago)* v.68(2) p.119-124; Apr 1991
Three systemic fungicides, flutolanil, triadimenol and hexaconazole, were evaluated in Trinidad against witches' broom disease of cocoa on young clonal plants and mature trees. Their effects on the growth of newly-formed cushion brooms and on basidiocarp production of dry brooms were also assesed. All formulations applied as pre-inoculation soil drenches to the young plants significantly

reduced infection by *Crinipellis perniciosa*. All fungicides also significantly suppressed a further growth of the cushion brooms. Flutolanil and triadimenol reduced the numbers of basidiocarps formed on dry brooms. The results are discussed with suggestions for strategies for future investigations on chemical control of the pathogen.

Speciality cocoa beans: a market to watch carefully
BYSKOV, B. and SCHEU, H. - *International Trade Forum (UNCTAD/GATT))* v.27(1) p.26-29; Jan 1991

There are 3 types of fine or flavour cocoa beans (Criollo, Trinitario and Nacional). The main supplier countries of these fine cocoa beans are listed including information on the percentage of fine cocoa of the total production. The fine cocoa is used in speciality chocolate and blended with ordinary cocoa. The main import countries are the USA, Germany, Japan, Switzerland, France and the UK in descending order of import volume. The world market prices of fine cocoa beans follow the overall world market prices. The premiums tend to be very small and in some countries these beans are even sold below the price of ordinary cocoa beans. The propects of increasing the use of fine cocoa are highlighted.

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Printed by Haines Clark & Co. Ltd., Gooch Street, Birmingham B5 7HE England.