

# **Sugarcane Germplasm Conservation and Exchange**

**Report of an international workshop held in  
Brisbane, Queensland, Australia  
28–30 June 1995**

*Editors*

**B.J. Croft, C.M. Piggin, E.S. Wallis and D.M. Hogarth**

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# Foreword

ANALYSIS by the Technical Advisory Committee of the Consultative Group of International Agricultural Research Centres (estimated that in 1987–88 sugar was the fourteenth most important crop in developing countries, with a gross value of production of over US\$7.3 billion. In Australia it is the third most important crop, with a value in 1994–95 of around A\$1.7 billion. The importance of sugar in developing countries and Australia makes it an appropriate focus for collaborative research and development with support from the Australian Centre for International Agricultural Research (ACIAR).

The importance of international collaboration for Australia is further emphasised by the fact that the Australian industry is based on imported germplasm; there are no native *Saccharum* species.

The Australian sugar industry has been cautious about supporting international scientific collaboration. Since 1982, ACIAR has funded only one collaborative project on sugar — evaluating policy options for sugar production and marketing in the Philippines. In recent times, however, industry keenness has grown and opportunities to promote such collaboration were discussed between ACIAR, the Sugar Research and Development Corporation and the Bureau of Sugar Experiment Stations in a series of meetings during 1994.

It was resolved that, because of the importance of germplasm exchange in the development of sugarcane industries worldwide and the recent findings of new virus diseases in germplasm centres and exchanged germplasm, a workshop should be held on safe management and international exchange of sugarcane germplasm.

This workshop, which is reported in these proceedings, was held near Brisbane in Queensland, Australia, on 28–30 June 1995. A committee consisting of Barry Croft, Mac Hogarth, Peter Whittle, Bob Dodman, Eoin Wallis and Colin Piggin organised the workshop. Ted Henzell also helped greatly with its organisation and running.

People from Australia (21) and overseas (14) attended, presenting papers and participating in discussions on a range of issues related to the collection, characterisation, conservation, cleanup and exchange of sugarcane germplasm. Consideration of such issues concerning vegetatively propagated species was enhanced through several case studies of successful operations internationally with banana at the International Network for Improvement of Banana and Plantain, in Australia with potato at the Institute for Horticultural Development Knoxfield, and in the United States with fruit trees at Beltsville, Maryland.

The excellent organisation, the spectacular and beautiful location of Clearview Mountain, and the enthusiasm and expertise of participants, all conspired to foster a very positive spirit at the workshop. It proved possible within the three days to review current knowledge and experiences with the exchange and quarantine of sugarcane and to develop action plans to address the major constraints. Outputs included recommendations for germplasm conservation, exchange, quarantine and conservation based on current knowledge, and the identification of priorities for future research and development, including opportunities for international collaboration.

Presented papers and a summary of outcomes from the workshop are presented in these proceedings. It is expected that these will provide background information to develop and seek support for a range of collaborative research, development and training projects on issues concerned with the conservation, exchange and use of sugar germplasm.

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6 September 1995

# Contents

Objectives of the Workshop <i>C.C. Ryan</i>	1
Importance of International Germplasm Exchange to the Australian Sugar Industry <i>D.M. Hogarth and N. Berding</i>	2
Review of Restriction to Free Access to Germplasm Exchange Facing Australian and Other International Sugar Industries <i>B.J. Croft</i>	6
<b>International Sugarcane Germplasm Collections</b>	
Role of ISSCT in Promoting Sugarcane Germplasm Collections and Their Maintenance <i>B.T. Egan</i>	12
The Role Of IPGRI in Germplasm Conservation <i>F. Engelmann and J. Engels</i>	14
Current Status of the World Sugarcane Germplasm Collection in Florida <i>J.C. Comstock, R.J. Schnell and J.D. Miller</i>	17
Conservation of Sugarcane Germplasm In India Given the Occurrence of New Viral Diseases <i>K.C. Alexander and R. Viswanathan</i>	19
<b>Major Diseases Affecting Sugarcane Production and Recent Experiences with Sugarcane Diseases in Quarantine</b>	
Major Diseases Affecting Sugarcane Production in the United States <i>J.C. Comstock</i>	24
Acquisition and Quarantine of <i>Saccharum</i> spp. Germplasm and Related Genera in the United States <i>S.S. Hurtt</i>	27
Major Diseases Affecting Sugarcane Production on the Ramu Sugar Estate, Papua New Guinea <i>S. Suma and E. Pais</i>	30
The Sugarcane Disease and Quarantine Situation in Southern Africa <i>R.A. Bailey</i>	34
Problems of Pests and Diseases for Sugarcane and the Resistance Breeding Program in Indonesia <i>P.D.N. Mirzawan, Irawan, and S. Lamadji</i>	36
Disease Constraints Associated with the Movement of Sugarcane Germplasm to and from Mauritius <i>S. Saumtally, S. Dhayan, S. Ganoo and S. Sullivan</i>	42
Major Diseases Affecting Sugarcane Production in India and Recent Experiences in Quarantine <i>K.C. Alexander and R. Viswanathan</i>	46
Major Diseases Affecting Sugarcane Production in Guadeloupe and Réunion Island, and Recent Experiences with Sugarcane Diseases in Quarantine at CIRAD-CA in Montpellier <i>P. Rott, J.H. Daugrois and J.C. Girard</i>	49
Major Sugarcane Diseases in Fiji <i>R. Tamanikaiyaroi and S. Johnson</i>	51
Major Diseases Affecting Sugarcane Production in Australia and Recent Experiences with Sugarcane Diseases in Quarantine <i>B.J. Croft and G.R. Smith</i>	55
Sugarcane Diseases in Southern Brazil: A Brief Report <i>W.L. Burnquist and J. Vega</i>	59

<b>Excluding Diseases in Exchange of Vegetatively Propagated Crops</b>	
Optimising Disease Expression in Quarantine <i>R.L. Dodman</i>	64
Role of In-Vitro Maintenance of Sugarcane for Germplasm Conservation and Exchange <i>J.C. Glaszmann, P. Rott and F. Engelmann</i>	67
New Technologies for Indexing Crops for Pathogens <i>J.W. Randles</i>	71
Molecular Tools for Indexing in Sugarcane <i>K.S. Braithwaite and G.R. Smith</i>	75
Indexing of Potato Tubers for the Presence of Ring Rot and Brown Rot <i>A.C. Hayward</i>	79
The Victorian Certified Seed Potato Scheme <i>J. Moran and R. Osborn</i>	83
The Safe Movement of <i>Musa</i> Germplasm <i>D.R. Jones</i>	85
Indexing and Establishing Disease-Free Germplasm of Pome and Stone Fruit Crops <i>S.S. Hurtt and H.E. Waterworth</i>	90
Detection of Latent Viruses and Double-Stranded RNAs in Clonal Grasses During Post-Entry Quarantine <i>K.C. Davis and M.R. Gillings</i>	95
<b>Major Quarantinable Diseases of Sugarcane</b>	
Pest Risk Analysis and Its Implications for Pest and Disease Exclusion in International Germplasm Exchange <i>S. Singh</i>	100
Sugarcane Bacilliform Virus, Sugarcane Mild Mosaic Virus and Sugarcane Yellow Leaf Syndrome <i>B.E.L. Lockhart, M.J. Irey and J.C. Comstock</i>	108
Downy Mildew, Ramu Stunt and Ramu Orange Leaf <i>R.C. Magarey</i>	113
Ratoon Stunting Disease <i>S. Saumtally, A. Dookun and L.J.C. Autrey</i>	116
Gumming Disease <i>S. Saumtally, A. Dookun and L.J.C. Autrey</i>	118
Sugarcane Mosaic and Fiji Disease <i>G.R. Smith</i>	120
Leaf Scald Disease <i>P. Rott</i>	123
Red Leaf Mottle <i>P. Rott</i>	125
Smut, Streak Virus and New Reovirus <i>R.A. Bailey</i>	126
<b>Workshop Summaries</b>	
Disease Risk Analysis and Strategic Planning for Conservation and Exchange of Sugarcane Germplasm <i>P.J.L. Whittle and P.H. Twine</i>	130

# Objectives of the Workshop

C.C. Ryan\*

MOST SUGARCANE INDUSTRIES around the world have been established as a result of the introduction of sugarcane from other regions. Quarantine has played an important role in restricting the spread of sugarcane pests and diseases around the world.

The recent discovery of several new diseases and the detection of some of these in quarantine and in germplasm collections necessitate a systematic review of the implications for future exchange and conservation of sugarcane germplasm.

The long term objective is to have implemented 'best practice' for germplasm conservation and exchange by all groups involved in international sugarcane germplasm conservation and exchange. This workshop planned a program to enable the exchange of germplasm with known disease status.

To achieve this, the workshop considered:

- quarantinable diseases inhibiting international exchange of sugarcane germplasm and the risks associated with these diseases; and
- the development of procedures for indexing and establishing disease-free sugarcane germplasm.

Having considered these issues the workshop developed plans for future directions for implementing best practices for international germplasm conservation and exchange. It is expected that this will lead to collaborative research projects.

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# Importance of International Germplasm Exchange to the Australian Sugar Industry

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## Abstract

The release of foreign germplasm to the Australian sugar industry has been severely reduced due to the finding of germplasm infected with sugarcane bacilliform virus, sugarcane mild mosaic virus or yellow leaf syndrome. Germplasm exchange is important for any sugar industry because it provides a source of clones for commercial production or for breeding purposes. Australia, in particular, has benefited greatly from the introduction of clones that have been grown commercially and from clones that are prominent in the cane breeding program. A system for free and safe exchange of modern cane hybrids is proposed.

SUGARCANE is not native to Australia, despite the centre of origin of several species in the genus *Saccharum* L. being the island of New Guinea, immediately off Australia's northern coast. Introduction of sugarcane germplasm to Australia was essential for the establishment of the industry. During the nineteenth century, several expeditions collected *S. officinarum* L. clones from native gardens in New Guinea to assess its potential for commercial production in Australia. Other *S. officinarum* clones were introduced from established sugar industries. While the introduction enabled the infant industry to become established, it also facilitated the entry of a range of sugarcane diseases because of a lack of quarantine.

In the twentieth century, Dutch breeders in Java, and Indian breeders, pioneered the development of *Saccharum* spp. hybrid clones for commercial production. This largely was in response to the susceptibility of *S. officinarum* clones to a range of important diseases, and their poor persistence under commercial cultural conditions. This development of *Saccharum* spp. hybrid clones led to a rapid replacement of *S. officinarum* clones in commercial cultivation, and heralded increased yield, higher sugar content, and improved disease resistance throughout the sugar industries of the world.

## Current Quarantine Problems

In recent years the release of foreign germplasm to the Australian sugar industry has been severely reduced for several reasons.

- The Bureau of Sugar Experiment Stations (BSES) decided not to release from quarantine any germplasm infected with sugarcane bacilliform virus and/or sugarcane mild mosaic virus.

- BSES quarantine found imported clones infected with yellow leaf syndrome.
- BSES is willing to accept only germplasm that is grown under acceptable disease-free conditions.
- Some countries are unwilling to exchange elite commercial germplasm.

A future demand on quarantine will be the introduction of large progeny populations for collaborative molecular and agronomic evaluation. This will limit Australia's capacity to introduce foreign germplasm for breeding purposes.

## Benefits of Germplasm Exchange to Australia

The major benefits of germplasm exchange for any sugar industry follow.

- High-yielding, disease-resistant *Saccharum* spp. hybrid clones can be introduced for commercial production.
- *Saccharum* spp. hybrid clones with particular attributes can be introduced for breeding.
- Basic germplasm from the *Saccharum* complex can be introduced for use in introgression programs.
- Export of Australian *Saccharum* spp. hybrid clones for offshore disease screening provides information on resistance to disease organisms currently found only in other countries — for example, smut and downy mildew. The value of such data can be discounted if the pathogenic organism displays variation for pathogenicity. Then the pathogen population used for resistance testing may differ genetically from that which eventually enters Australia, so that resistance ratings are not relevant.

## Introduction for commercial production

The *S. officinarum* clone Badila was introduced from New Guinea in 1896 and rapidly became the major cultivar in Queensland. Badila is still approved for planting in one Queensland sugar mill area, largely for sentimental reasons.

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The clone, NCo310, introduced from South Africa, was the major clone in Queensland for about 20 years, and reached a peak of 35% of the total crop. Until 10 years ago, most of the southern Queensland crop was produced from introduced clones. Introduced clones are still of importance in the northern New South Wales industry.

The important contribution of introduced clones to the Queensland industry is summarised in Table 1. From 1973 the proportion of the crop produced by imported clones ranged from a peak of 38% in 1977 down to 8% in 1994. This impact of imported clones in the commercial scene, while giving testimony to the unbiased assessment received by introduced material, largely reflects the inadequacy of resources directed to plant improvement programs in the southern regions of the Queensland industry. Plant breeders in BSES believe that introduced clones will never contribute to this extent again, largely because of the improved programs being conducted. However, introduced clones may satisfy particular clonal niches in the industry. If a new disease enters the industry, introduced clones with resistance could well prove important. For example, if smut became endemic, adapted Australian clones with resistance could take several years to select and place into commercial production. If BSES can maintain an active introduction program, overseas clones with resistance would be available and could be used in an emergency situation.

#### Introduction for breeding purposes

The most important reason for the Australian sugar industry maintaining a germplasm exchange program is to provide germplasm with desirable traits for the breeding programs. Most introduced clones will not be adapted to Australian conditions, but most have traits of benefit to the Australian sugarcane improvement programs. These traits may be cane yield, sugar content and disease or insect resistance.

Berding and Skinner (1987) discussed the important question of genetic diversity in commercial crop production, and considered the parentage of commercial clones from Q80 to Q144. This consideration is updated in Table 2. Here, parentage was considered in five

**Table 1.** Tonnage<sup>a</sup> and percentage of the Queensland sugarcane crop produced by clones of foreign origin, 1973 to 1994.

Year	Cane production (tonnes)	Proportion of crop (%)
1973	5 297 227	28.9
1974	6 469 023	33.3
1975	7 240 661	34.4
1976	8 070 243	36.3
1977	8 493 883	38.0
1978	6 940 619	34.5
1979	6 350 197	32.1
1980	6 950 569	30.8
1981	7 221 374	30.6
1982	6 687 502	29.0
1983	6 298 328	27.7
1984	7 261 591	30.3
1985	6 962 161	30.3
1986	7 974 967	33.1
1987	6 687 070	27.9
1988	6 586 641	24.9
1989	6 040 060	23.1
1990	4 504 803	18.8
1991	3 102 764	15.6
1992	3 242 896	11.8
1993	2 794 620	9.2
1994	2 623 129	8.0

<sup>a</sup> Data were taken from the annual reports of the Bureau of Sugar Experiment Stations, Queensland, for the years 1974 to 1995, inclusive.

**Table 2.** Contribution of foreign clones to the parentage of five groups of 17 commercial clones released by the Bureau of Sugar Experiment Stations since the introduction of controlled cross-pollination, the number of known parents in each group, and a measure of deviation of parental composition of each group from that of all the clones.

Clonal group	No. of foreign clones in parentage of each clone			No. of known parents	$\chi^2$ <sup>a</sup>
	0	1	2		
Q79-Q95	1	16	0	31	0.33
Q96-Q112	3	12	2	34	0.01
Q113-Q129	3	11	3	34	0.20
Q130-Q146	3	12	2	34	0.01
Q147-Q163	8	6	1	30	3.24

<sup>a</sup>  $\chi^2_{(1, 0.05)} = 3.84$ . The observed number of foreign and domestic clones within each group was compared with the expectation computed over all groups: no. of foreign clones = 73; no. of domestic clones = 90. The number of accounted parents (163) is seven fewer than expected ( $5 \times 17 \times 2 = 170$ ). Three clones in the Q79-Q95 group resulted from polycrosses, and the male was unknown. Two clones in the Q147-Q163 group resulted from 'mixed seedlings', where parents were unknown.



groups of 17 commercial clones from Q79 to Q163. In each group except the most recent (Q147–Q163), the majority of clones had at least one foreign clone as a parent. A chi-square test for the observed number of foreign and domestic clones in the parentage of each group, against expectations from all 85 clones, approached significance only for the last group (Q147–Q163, Table 2). This shift to use of domestic parental clones for production of commercial clones for the Australian industry reflects greater emphasis on recurrent use of these clones. As well, there has been a reduction in the vigour with which foreign germplasm is used in the programs.

The composition of the active parental collection available at BSES Meringa for use in cross-pollination in 1995 is detailed in Table 3. Only 14% of the collection is foreign germplasm, while BSES produced clones account for 79% of the collection. The current composition of the BSES parental collection, and the use of foreign germplasm, are probably doing little to improve the genetic diversity in the commercial crop in Australia. A molecular analysis of genetic diversity at the commercial level, as performed by Harvey et al.

**Table 3.** Composition, by clonal source, of the active parental collection available at BSES Meringa for use in cross-pollination in 1995.

Clonal source	No.	%
Foreign	283	14.3
Domestic		
BSES <i>Saccharum</i> spp. hybrids	1 559	78.9
Q	47	
Northern	839	
Herbert	39	
Ayr	141	
Central	253	
Southern	240	
BSES introgression	33	1.7
CSR	101	5.1
<b>Total</b>	<b>1 976</b>	

**Table 4.** Range and mean age of seedling year, and range and mean of years present in the Meringa collection, for foreign clones and BSES clones originating from the Meringa (N) and Bundaberg (S) programs available for cross-pollination in 1995.

Clonal source	No.	Seedling year		Years in collection	
		Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
Foreign	255 <sup>a</sup>	1951–1983	1969 $\pm$ 6	1–13	7 $\pm$ 3
BSES – N	839	1965–1991	1982 $\pm$ 4	1–13	6 $\pm$ 3
BSES – S	240	1959–1989	1981 $\pm$ 4	2–13	5 $\pm$ 2

<sup>a</sup> Only 255 of the 283 clones introduced from foreign programs, present in the active parent collection at Meringa for 1995, could be dated as to seedling year from data available.

(1994) for the South African crop, would be most revealing, and perhaps frightening.

An examination of the age structure of the foreign clones in the BSES parental collection is revealing (Table 4). Only 255 of the 283 clones in this category could be dated, because not all clonal identifiers contain a year element, and other data were not available. The average year of seedling origin of foreign germplasm was 1969. This contrasted markedly with the average year of seedling origin of parental clones that were produced in BSES programs at Meringa (1982) and Bundaberg (1981). On average, the foreign clones are 13 years older than the group of parental clones produced at Meringa. This is almost twice the interval from seedling initiation until completion of yield trial assessment that allows new clones produced at Meringa to be used as parents. Some delay in using introduced germplasm must be expected, as quarantine and propagation barriers must be negotiated. However, the conclusion that BSES's current foreign germplasm collection is dated, on average, is inescapable.

The average residency in the collection at Meringa varied little for these three classes, being 7, 6, and 5 years, respectively (Table 4). Because of this, it can be concluded that a long acquisition time from the time foreign commercial clones are produced is primarily responsible for the relatively old age structure of this segment of the parental collection. An examination of germplasm exchange arrangements with collaborating countries to resolve this issue would seem timely and would be mutually beneficial, provided that quarantine impediments could be resolved.

### Basic Germplasm

The BSES breeding program still has a requirement to introduce material of this classification. This was evident in 1994, when high-sucrose *S. officinarum* clones were sought from the collection maintained by the Indian Sugarcane Breeding Institute at Kannur in Kerala. This operation, funded by the Australian Sugarcane Research and Development Corporation, with access to the World Collection sanctioned by the Indian Council of Agricultural Research, sought to expand

the pool of *S. officinarum* available in Australia. Emphasis was on introducing 200 clones with the highest sucrose level from the approximately 750 available. The objective was to commence a breeding program to produce a new generation of clones with improved sucrose content. This project was terminated by the occurrence in these clones in Brisbane quarantine of a putative outbreak of an unknown virus. This event adds substance to the necessity for this workshop. The desire to introduce relatively large numbers of clones, as in this project, is undiminished on the part of BSES breeders. The feasibility of satisfying this will be low until the quarantine aspects of such movements can be resolved satisfactorily.

### Industry Attitude

The Australian sugarcane industry probably is no less conservative than any agriculturally based industry elsewhere in the world. Germplasm exchange can be a sensitive issue, with the liberal attitude of the breeding team being continually questioned. 'Why should we give away our latest Q cane?' is an often asked question. In the automotive industry in the United States the competitive advantage conferred from the introduction of a technical innovation is considered to last for only 14 months. By contrast, in Australia's agricultural situation, the earliest one can conceive of commercially exploiting an introduced clone would be 10 years after receipt in BSES quarantine. This timing should apply to the reverse situation. A vigorous sugarcane improvement program can make significant advances in this period. The competitive position of any sugarcane industry maintaining well funded plant improvement programs cannot be jeopardised by advocating and participating in a vigorous germplasm exchange program.

The industry has benefited tremendously from access to foreign clones for commercial production (Table 1). Tew (1987) listed major clones in production in 51 sugar industries. Only one BSES produced clone (Q83) had acquired major status outside Australia, and accounted for 14.3% of the 1984 crop in Thailand. As well, the Australian industry has benefited significantly from the contribution foreign germplasm has made to the parentage of domestically produced

commercial clones (Table 2). Obviously, by these two measures, the industry has enjoyed significant benefits by engaging in an open and vigorous exchange program. Regrettably, this will be jeopardised unless the current quarantine impasse can be resolved.

### Vision for the Future

The vision for the future is free and safe exchange of modern varieties. It is suggested that countries (or organisations) involved in the exchange of germplasm put 5–10 clones from late-stage selection trials each year into quarantine. The clones should be indexed for all important diseases. After two years in quarantine, the clones would be freely available to all cooperating countries, and they would remain available for three years. At the end of three years, they would be removed from quarantine and would no longer be available.

The proposed process would ensure that recently selected clones would be available for free and safe exchange. The risks currently associated with imported foreign clones would be greatly reduced, and breeders would be able to use the best hybrid clones from around the world in their breeding programs. All cane breeding countries would benefit.

### Acknowledgment

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# Review of Restrictions to Free Access to Germplasm Exchange Facing Australian and Other International Sugar Industries

B.J. Croft\*

## Abstract

At least ten new sugarcane diseases of significance to quarantine have been reported in the past ten years. A pest risk analysis that includes these diseases should be conducted if germplasm is to be imported from countries that have reported these diseases. In some cases little is known of the causal agent and/or the biology of the new diseases. This means that the pest risk analysis may suggest limiting imports from infested areas until further information is available. The research required to satisfy the pest risk analysis may require considerable long term funding. Research into the new sugarcane diseases will require an international cooperative exercise.

IMPROVED EFFICIENCY in germplasm exchange could be achieved if a database listing the availability and the attributes of clones were available. The preservation of the world collection of basic germplasm should be a high priority for all sugarcane industries.

Germplasm is quarantined primarily to prevent the introduction of pests and diseases or strains of a pathogen from entering a country where they either do not occur or are under active control. Germplasm exchange in sugarcane has played a major role in the development of sugar industries worldwide through the direct commercial use of cultivars bred in other countries and the use of imported germplasm for breeding purposes. Before the early twentieth century, sugarcane was introduced to many countries with minimal concern about transferring diseases or pests (Hughes 1960). Consequently some diseases — for example, leaf scald and mosaic — occur in most sugarcane producing countries. Diseases with few external symptoms such as ratoon stunting disease were transferred around the world until their discovery and diagnostic procedures were developed. In spite of the extensive transfer of sugarcane germplasm before quarantine procedures were introduced, some important diseases are limited in distribution.

In recent years a number of previously unidentified sugarcane diseases that have significance for quarantine have been recorded. These include bacteriosis (Anon. 1995), red leaf mottle (peanut clump virus — Baudin and Chatenet 1988), yellow leaf syndrome (Borth, Hu and Schenck 1994), sugarcane bacilliform virus (Lockhart and Autrey 1988), sugarcane mild mosaic virus (Lockhart, Autrey and Comstock 1992), Ramu streak (Magarey, Suma and Egan 1995), Ramu stunt (Walker, Egan and Eastwood 1987), Ramu

orange leaf (Magarey et al. 1995), a new potyvirus from Pakistan (Jensen and Hall 1993) and a new reovirus in South Africa (Bailey, these Proceedings, pp. 126–127). All but two of these diseases are suspected or known to be caused by viruses. As well as these new diseases, unusual symptoms have been detected in the canes held in quarantine in Taiwan (sereh-like symptoms in a clone from China), Australia (yellow-red leaf fleck in noble clones from India and Hawaii) and Mauritius (red leaf mottle in clones from Canal Point, Florida). The uncertainty caused by the reports of these previously unidentified diseases has greatly restricted the movement of sugarcane germplasm and was instrumental in the convening of the International Workshop on Sugarcane Germplasm Conservation and Exchange.

## Pest Risk Analysis

The finding of any new disease in a country exporting germplasm raises concern about continued access to germplasm from that country. A pest risk analysis (Phillips, Chandrashekar and Roberts 1994) should be conducted to answer the following questions before further imports of germplasm take place.

### *The disease*

1. What is the causal agent? Are there different strains of the disease agent?
2. Does the disease agent already occur in the importing country or is it under active control?
3. What is the basic biology of the disease agent? How does it spread? How widely distributed is the disease and how rapidly does it spread? What survival propagules are formed? What alternative hosts will it infect? Are these hosts important crop species?
4. How serious a threat does the disease pose to the sugar industry and other plant industries?

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5. Does the disease show diagnostic symptoms under quarantine conditions?
6. Are eradication treatments available to free infected germplasm from the disease?

#### *The exporting country*

1. What precautions does the exporting country take to ensure exported germplasm is not exposed to the disease? Is the germplasm being exported as seed, stalks or in-vitro plantlets? Has the germplasm been indexed for the disease?
2. How widely distributed is the disease?

#### *The importing country*

1. Does the importing country have containment or isolation facilities? Do staff in the importing country have sufficient expertise to detect symptoms of the disease?
2. Are diagnostic assays available and does the importing country have the expertise to conduct these assays? What is the availability of antisera or DNA primers for indexing assays?

#### *The germplasm*

1. How important is the germplasm compared with the risk of entry of the disease? Can the germplasm be sourced from another country without the disease?

To answer all of these questions for one disease may require considerable research. Currently sugarcane quarantine facilities are faced with asking these questions for up to ten new diseases. It should be noted, however, that a low risk rating for one question may greatly reduce or remove the risk and therefore make answering the other questions a low priority. For example, if the germplasm is not of high value it may be decided to stop imports or if a reliable method of eliminating the disease is available the risk is greatly reduced. In some cases, even with diseases that have been recognised for many years, the answers to the above questions are not known and judgments have to be made about the risks compared with the potential gains from importing the germplasm. When all available information is gathered and assessed a decision is then made either to continue importations or to cease any further importations indefinitely or until further information becomes available. Because of the value of imported germplasm it is unlikely that countries will block imports of germplasm for long periods from key suppliers unless there is a high risk with no means of reducing that risk.

### **An Example — Yellow Leaf Syndrome**

Yellow leaf syndrome was first identified in Hawaii in 1989 (Borth et al. 1994) and has subsequently been reported from Brazil (Centro De Tecnologia Copersucar 1993) and Florida (Comstock, Irvine and Miller 1994). From the Australian sugar industry perspective

the answers to the above questions for yellow leaf syndrome are as follows.

#### *The disease*

1. The causal agent is unknown. It is suspected that a luteovirus may be involved. The existence of strains is unknown.
2. Similar symptoms to yellow leaf syndrome have been observed in fields throughout Queensland. Inspectors have had limited experience with the disease and are not confident that the disease can be positively identified from symptoms alone. Yellow leaf syndrome may already be present but this cannot be confirmed.
3. The basic biology of the disease agent is not known. Experiments that demonstrated disease spread have been conducted in Hawaii.
4. Reports from Hawaii and Brazil suggest extensive yield losses in susceptible cultivars. Important commercial cultivars have been discarded because of the disease.
5. Yellow leaf syndrome expresses symptoms in the cooler months, and under glasshouse conditions symptoms may be masked. Recent experience in Australia suggests that some clones will express symptoms in quarantine glasshouses.
6. No information is available about eradication except that hot water treatment (50°C for 3 hours) does not eliminate the disease (Borth et al. 1994).

#### *The exporting country*

1. Exporting countries were unaware of the presence of the disease until recently and therefore have taken no precautions to limit exposure of export canes to the disease. The germplasm is exported as stalk cuttings.
2. The disease appears to be widely distributed in Brazil and the United States.

#### *The importing country*

1. Excellent containment facilities are available in Australia at the Bureau of Sugar Experiment Stations in an area isolated from commercial cane growing districts. Quarantine inspectors require more training and experience with the disease.
2. Specific assays for yellow leaf syndrome are not available. Non-specific assays such as dsRNA tests and generic luteovirus probes may be useful but the results may not be conclusive. Electron microscopy is difficult for low titre viruses such as luteoviruses. The expertise for conducting these tests is available but is not currently used routinely on quarantine canes.

#### *The germplasm*

1. Germplasm from Florida, Hawaii and Brazil would be considered of very high value to the Australian sugar industry.

Considering this information the Bureau of Sugar Experiment Stations, in consultation with Australian Quarantine and Inspection Service (AQIS), has limited imports from these countries until further information is available about the disease.

### Research into New Diseases

Obviously considerable research is required to answer the many questions associated with a newly described disease. Until this research is conducted the lack of information will be a major factor in any decisions made about the risk from a disease.

In some cases considerable research is required to develop the basic skills for diagnosis so that further research can be conducted. Once these skills are developed there is a need to ensure that basic biology questions on, for example, the distribution of the disease agent, transmission and the effect of the disease on yield are answered so that the risk analysis can be based on reliable information. This requires a long term commitment to research. For example, detailed molecular information is available about the sugarcane bacilliform virus but the effect of the virus on the growth of cane has not been studied in detail. Until this information is obtained it will be difficult to fully assess the implications of the sugarcane bacilliform virus for sugarcane production and quarantine.

In many cases the exporting country will have different priorities for funding research into the new disease from the importing country and funds for research into the disease may not be available in the exporting country. Research into exotic diseases in the importing country may be undesirable due to the risk of escapes. Even if funds are available, few countries can afford to carry out research into a large number of new diseases. Extensive cooperation between countries will be required to assess the risks associated with the recently identified sugarcane diseases.

An example where international cooperation is required to address a disease risk is the serious disease, Ramu stunt, which occurs in Papua New Guinea (Magarey et al. 1995), the centre of origin of *Saccharum officinarum*. This disease can cause severe losses but the small sugar industry in Papua New Guinea cannot fund research to fully characterise the causal agent even though it has funded some research into the biology and control of the disease. It would be in the interest of all sugarcane producing countries to assist with funding research into this devastating disease. Any future collections of basic *Saccharum* germplasm from Papua New Guinea would need to be carefully screened for Ramu stunt.

### Maintenance of Germplasm and Expertise

Even if funds are available to develop diagnostic tests and procedures to eradicate important disease agents

from germplasm there is need for an ongoing commitment. Ongoing funding is required to set up and maintain a testing and eradication laboratory. The tests for a wide range of diseases could be conducted in a centralised laboratory or germplasm could be tested in different laboratories around the world where expertise in the particular disease resides. Facilities and staff would also be required to maintain the indexed germplasm in a disease-free state. In-vitro germplasm conservation is already used for sugarcane exported by CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement), France, and it may offer significant advantages in maintaining germplasm with low risk of reinfection with diseases (International Board for Genetic Resources 1988).

### Characterisation of Germplasm

Because the indexing, eradication of disease agents and maintenance of germplasm in a disease-free state is likely to be expensive the number of clones that could be handled would be restricted. This would mean that better characterisation of germplasm would allow a more efficient use of resources. New DNA fingerprinting and marker technology may provide the tools so that informed decisions can be made about the value of different clones for breeding purposes.

### Loss of Genetic Diversity

In the past *Saccharum* and related genera have been collected internationally from the wild. The germplasm collected has been widely distributed, two major collections being in India and Florida. Maintaining or re-establishing the integrity of the existing collections should be viewed as a high priority for international sugarcane industries.

The wild populations of *Saccharum* and related genera are not under immediate threat but as the economies develop in countries with wild populations of these genera some species or clones may disappear. The situations in these countries should be monitored so that action to preserve basic germplasm can be taken before stocks are lost. The existence of serious yet unidentified diseases in these wild populations would seriously limit access to these sources of germplasm. Skills for detecting unidentified diseases, particularly viruses, would be required for indexing future collections of wild germplasm.

### Administrative Issues

The policies on exports of germplasm vary by country and may limit access to germplasm. Policies range from unrestricted access to very limited access to germplasm. Some countries will exchange germplasm only with countries that have bona fide breeding programs and can therefore offer valuable material in

exchange. The export of germplasm, particularly large numbers of clones, may be an expensive exercise and some countries will require financial or physical assistance to arrange it.

Another administrative limit to access to germplasm is the lack of a database listing the availability of clones in different countries. Information on the attributes of the available clones would greatly increase the value of a database. Again the development of this database would require an international cooperative exercise.

### Conclusions

During the past ten years at least ten previously unidentified diseases of sugarcane have been recorded, some of which have caused extensive losses in the countries where they have been reported. For many of the diseases little is known about the causal agent and/or the basic biology of the disease.

The lack of knowledge about these new diseases is currently the greatest barrier to free access to sugarcane germplasm. To overcome this lack of knowledge requires extensive research that is beyond the funding capacity of individual countries and requires a cooperative approach from the major sugarcane producing countries. Once the basic information is available an ongoing commitment will be required to maintain the expertise and facilities required to index germplasm, eradicate any diseases detected and maintain it in a disease-free state.

Improved access to the information about availability of germplasm and better characterisation of important germplasm collections would improve the efficiency of germplasm exchange. The world sugar industries should examine systems in place for other vegetatively propagated crops such as potatoes and bananas to design improved procedures for conserving and exchanging sugarcane germplasm.

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# **International Sugarcane Germplasm Collections**

# Role of ISSCT in Promoting Sugarcane Germplasm Collections and Their Maintenance

B.T. Egan\*

## Abstract

The International Society of Sugar Cane Technologists pioneered the collection and conservation of sugarcane germplasm through its dedicated individual and organisation members. Its Germplasm Committee continues to play an important role in providing the forum and impetus for international cooperation on promoting and maintaining sugarcane germplasm collections.

THE INTERNATIONAL SOCIETY OF SUGAR CANE TECHNOLOGISTS (ISSCT) has had a long standing interest in the collection and conservation of sugarcane germplasm. This dates back to the Second ISSCT Congress held in Cuba in 1927, when a committee of relevant technologists was appointed with a charter to collect and conserve noble and wild canes.

## The Past

Some sugarcane research organisations were already involved in collection and conservation, but ISSCT provided the forum and the opportunity for greater international cooperation in all facets. Collections from centres of origin were promoted, and were held primarily in Florida (USA) and Java (then the Dutch East Indies). These became known in the sugarcane world as the ISSCT world collections.

Unfortunately, the Javan collection was lost in the 1940s during World War II. By the 1950s, plant breeders and research organisations had become concerned at the narrow genetic base of commercial sugarcane and the possible loss of valuable wild germplasm.

This was addressed at the Eighth ISSCT Congress in 1953, which produced an action plan to establish a second collection, encourage expeditions to unexplored areas, and establish a fund to characterise germplasm.

These issues were actively canvassed by the Germplasm Committee with considerable success. Firstly, India offered to expand its national germplasm collection at Coimbatore, and this was recognised as an ISSCT world collection at the Ninth Congress in 1956. Secondly, ISSCT member organisations were encouraged to collect germplasm in many strategic areas, often under the aegis of ISSCT, and many clones of sugarcane and related genera have been added to the world collections. Finally, an international levy on sugar production was proposed to provide adequate

funding for characterisation of the germplasm, but this failed to receive sufficient governmental support and lapsed; it has never been revived.

The ISSCT Standing Committee on Germplasm has continued to take an active interest in these and other related issues. It has acted as a repository and clearing house for information; promoted better morphological descriptors; recommended priority areas for collections; consulted with IBPGR (International Board of Plant Genetic Resources) on the collection, conservation and documentation of sugarcane germplasm; supported the efforts in the United States to cryopreserve true seed of *Saccharum* and other species as a backup for the world collections; and promoted debates on where to go next.

A collaboration began in 1981 with IBPGR, which previously had not been involved with sugarcane because of ISSCT's role. A sugarcane working group of ISSCT members, at an IBPGR-convened meeting, produced a set of key points that needed to be addressed. For various reasons, there was little further action in promoting greater IBPGR involvement with sugarcane.

ISSCT finances are limited, but in 1984 it introduced a series of sectional workshops for ISSCT members, held halfway between Congresses and with airfare subsidies for attendance of sectional committee members. The Germplasm Committee has held four well-attended workshops so far, providing greater opportunities for discussion and interaction, and laying the groundwork for current initiatives.

## The Present

The ISSCT Technical Coordination Committee recommended in 1991 that an international network of *Saccharum* germplasm collections be established under IBPGR, with ISSCT collaboration. The ISSCT Germplasm Workshop in 1991 supported this, but considered that existing collections needed to be documented and reviewed before a cogent, documented case could be put to IBPGR.

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The 1994 ISSCT Germplasm Workshop had the theme 'Germplasm Preservations — Accomplishments and Prospects'. An action plan was developed to address the major issues (see box 1).

**Box 1.** Action plan developed by the ISSCT Germplasm Committee at Montpellier Workshop, March 1994.

1. Improve communications between all sugarcane breeders.
2. Complete an inventory of *Saccharum* complex germplasm worldwide.
3. Obtain adequate resources for world collections, and consider their location.
4. Rationalise and coordinate world collections:
  - standardise molecular markers and other descriptors to be used; and
  - allocate responsibilities and time frames.
5. Establish an interface with IPGRI for network development:
  - seek initial meeting; and
  - establish a working group.
6. Designate a core collection.
7. Solicit and formalise institutional commitments for network components.
8. Promote the application of in-vitro storage techniques and use of seed storage conservation.

A representative of IPGRI (International Plant Genetic Resources Institute, the successor to IBPGR) outlined its philosophy on germplasm networks, and how IPGRI could assist.

The ISSCT Standing Committee on Germplasm is currently progressing these matters and reported on progress at the ISSCT Congress in Colombia in September 1995.

### The Future

ISSCT itself has neither the resources nor organisational structures to finance and coordinate the research and development needed to carry this work forward.

No small group of organisations, funded mostly by their national sugar industries, can be expected to fund all the work required because the whole world sugar industry will benefit.

Sugarcane is not only an industrial crop, but a far more valuable direct food crop than was previously thought. This should be an incentive for world aid agencies and IPGRI to become involved in germplasm preservation, in a joint effort with the industrial crop sector.

ISSCT and its Germplasm Committee should — indeed must — continue to provide the forum and impetus for international cooperation on all of these matters until a better system can be devised and introduced.

# The Role of IPGRI in Germplasm Conservation

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## Abstract

The International Plant Genetic Resources Institute, which is responsible for advancing the conservation and use of plant genetic resources, is active in undertaking research and developing strategies for seed conservation, in-vitro conservation, germplasm health and genebank management. As part of this it is involved in conserving sugarcane genetic resources and it recognises the importance of establishing a network of such resources.

THE INTERNATIONAL PLANT GENETIC RESOURCES INSTITUTE (IPGRI), formerly the International Board for Plant Genetic Resources (IBPGR), is an autonomous international scientific organisation operating under the aegis of the Consultative Group on International Agricultural Research. IPGRI's mandate is to advance the conservation and use of plant genetic resources for the benefit of present and future generations (IPGRI 1994). IPGRI works in partnership with other organisations, undertaking research and providing training and scientific and technical advice and information. There are four strategic objectives in its work on plant genetic resources: strengthening national programs, contributing to international collaboration, improving strategies and technologies for conservation and providing an international information service.

IPGRI has its headquarters in Rome, Italy, and regional offices for Sub-Saharan Africa; West Asia and North Africa; Asia, the Pacific and Oceania; the Americas; and Europe. The regional groups are responsible for developing regional strategies, assisting with national and regional programs and working on issues of regional significance. The three thematic groups — Genetic Diversity; Germplasm Maintenance and Use; Documentation, Information and Training — which are located at the headquarters develop and coordinate research and information services of interregional relevance in their respective subject areas, and provide scientific and technical support to the regional groups.

The aim of this paper is to give a brief overview of IPGRI's activities in plant genetic resources conservation and to present its past and present involvement in the conservation of sugarcane germplasm.

## IPGRI's Activities in Plant Genetic Resources Conservation

The Germplasm Maintenance and Use Group is responsible for the institute's activities in germplasm conservation. These activities include seed conservation,

in-vitro conservation, germplasm health, genebank management and the development of strategies for germplasm conservation and use.

### Seed conservation

The principal aims of IPGRI's research on seed conservation are:

- to advance understanding of the nature of seed desiccation and develop ways of overcoming problems in the conservation of recalcitrant seeds; and
- to develop low-input techniques to dry and store orthodox seeds.

Research into desiccation sensitivity and the nature of recalcitrance is pursued as part of the group's efforts on in-vitro technology. Orthodox seed research in IPGRI focuses on determining optimum seed moisture for storage and investigates ultra-dry seed storage. Other projects concern the detection of pathogens in seeds and the effect of pathogens on seed viability.

The range of species requiring *ex situ* conservation has broadened from major crops to include forestry species, and wild and underutilised species. Evidence for the storage behaviour of many of these species is either scattered in the literature or nonexistent. IPGRI has commissioned the production of a compendium of seed storage behaviour and is supporting research for developing a protocol for the determination of seed storage behaviour.

Finally, various projects address practical methods for processing and storing seeds in genebanks, including notably the use of silica gel for drying seeds, the effect of sun and shade drying on seed longevity and on the incidence of seed-borne diseases, the determination and monitoring of seed moisture content, and the performance of storage containers.

### In-vitro conservation

Research in this area focuses on the development of in-vitro conservation techniques for recalcitrant seed and vegetatively propagated plant species. Research on recalcitrant seeds presently includes the development of cryopreservation techniques for embryos and embryonic axes of several Dipterocarp species and

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tropical fruit trees, as well as the development of in-vitro techniques for collecting *Dipterocarp* germplasm in field conditions.

Several projects focus on in-vitro conservation of vegetatively propagated species. Cryopreservation techniques have been developed for apices of potato, sugarcane, banana and plantain. A project on in-vitro slow growth storage of sweet potato is being prepared. Finally, research on genetic stability is being pursued through a project aiming to evaluate the possibility of using random amplification of polymorphic DNA to detect off-types of banana and plantain generated in vitro and to monitor genetic stability of plant material during in-vitro storage.

### **Germplasm health**

IPGRI's involvement in germplasm health addresses technical, information and regulatory aspects.

Several research projects aiming to develop new methods of pathogen detection and therapy, and to adapt methods to circumstances in developing countries are under way. Information on the distribution, importance and handling of pathogens is regularly gathered and communicated to International Agricultural Research Centres (IARCs) and national genebanks by publications (for example, Technical Guidelines for the Safe Movement of Germplasm), presentations at international meetings and participation in training courses. Finally, IPGRI assists national programs, particularly quarantine authorities, in developing, updating and/or improving quarantine procedures.

### **Genebank management**

The objectives of IPGRI's research on the management of genebanks is directed at improving its efficiency and effectiveness in conserving genetic resources and making them available to users. Present activities include developing a seed genebank design program that allows genebank managers to plan requirements for the storage of a given species. Guidelines that present regeneration procedures for different categories of species are being developed. Operations of a number of genebanks are being studied, thus revealing key steps in the management decision pathway.

### **Strategies for germplasm conservation and use**

Research on strategies for conserving and using plant genetic resources aim to improve the overall sustainability of the conservation efforts through greater security and economy of conservation, and enhanced accessibility and use of germplasm.

In this aim, IPGRI addresses different aspects and dimensions of genetic resources conservation and use, including:

- the development of technologies and procedures for conservation and use;
- the development and application of the principle of complementary conservation strategies;
- the organisation and management of genetic resources conservation through assistance to national programs and the establishment and operation of genetic resources conservation networks; and
- involvement in discussions on international arrangements and policy issues for genetic resources conservation.

### **IPGRI's Involvement in the Conservation of Sugarcane Germplasm**

IBPGR has been involved in sugarcane genetic resources conservation activities since 1978. IBPGR supported studies on sugarcane seed storage (Ellis 1983; IBPGR 1984). In 1981 IBPGR convened a small working group of experts to advise on the collection, conservation and documentation of sugarcane germplasm (IBPGR 1982). An inventory of sugarcane germplasm collections was made during the meeting and published by IBPGR (Williams and Damiana 1981). IBPGR participated in implementing the action plan set up by the working group. IBPGR designated the two ISSCT (International Society of Sugar Cane Technologists) germplasm field collections in Coimbatore, India, and Canal Point, United States, and included them in the IBPGR network of field genebanks. Official agreement was obtained in 1983 with the United States (IBPGR 1984) and in 1988 with India (IBPGR 1989). The National Plant Germplasm System (United States) and the National Institute of Agricultural Research (Tsukuba, Japan) were designated as base seed collections for sugarcane (IBPGR 1984, 1987).

Following the assessment of taxonomical and geographic gaps performed during the 1981 meeting, priority areas for collecting were listed. IBPGR sponsored collecting missions in India, Thailand and the Philippines (Srinivasan, Palanichamy and Koppa 1982; IBPGR 1984, 1985). The reliability of IBPGR passport and management descriptors was tested with sugarcane in Barbados (IBPGR 1984). IBPGR also supported characterisation studies of the Caribbean collection and at the Florida Sugarcane League, United States (IBPGR 1987, 1988, 1989).

More recently, IBPGR and the FAO (Food and Agriculture Organisation of the United Nations) published technical guidelines for safely moving sugarcane germplasm (Frison and Putter 1993). In 1994 IPGRI supported a research program on the development of cryopreservation techniques for sugarcane, in collaboration with the Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM) and the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD, France) and the Centro Nacional de Investigaciones Científicas (CNIC, Cuba).

Following a recommendation of the ISSCT workshop 'Germplasm Preservation — Accomplishments

and Prospects', which took place in March 1994 in Montpellier, France, IPGRI has started interacting with ISSCT on establishing a germplasm conservation network for sugarcane.

### Conclusions

IPGRI is willing to provide assistance in establishing a sugarcane genetic resources network. IPGRI could give advice on the rationalisation of long term conservation combining field genebanks and a back-up system in vitro, and help in identifying bottleneck problems. IPGRI could assist the network in developing research projects and in identifying funding sources. Finally, IPGRI would like to stress the importance of developing projects combining germplasm conservation and use activities.

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# Current Status of the World Sugarcane Germplasm Collection in Florida

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## Abstract

The USDA-ARS Subtropical Horticultural Research Station in Miami, Florida, maintains the World Germplasm Collection of Sugarcane and Related Grasses. This collection consists of approximately 1500 *Saccharum* and related grass species. The damage to the collection in 1992 as a result of a hurricane highlighted the need to back-up inventories of sugarcane germplasm.

THE WORLD GERMLASM COLLECTION of Sugarcane and Related Grasses consists of approximately 1500 *Saccharum* and related grass species and has been maintained at the US Department of Agriculture, Agricultural Research Service, Subtropical Horticultural Research Station in Miami, Florida, since 1976. Prior to that the collection was maintained at Beltsville, Maryland, in greenhouses and before that at the USDA-ARS Sugarcane Field Station at Canal Point, Florida. Currently, the USDA-ARS plans to close the Subtropical Horticultural Research Station in Miami and move the World Germplasm Collection (except for the *S. spontaneum* clones) to an area leased by the USDA-ARS from the University of Florida, Agricultural Research and Education Center at Ft Pierce, Florida. This location was selected because it is approximately the same distance from commercial sugarcane as the Miami location is and has a similar climate. The *S. spontaneum* clones of the collection will be moved to the USDA-ARS Sugarcane Field Station at Canal Point where the individual clones will be grown in containers to prevent their spread by rhizomatous growth. This decision was based on the fact that *S. spontaneum* is classified as a noxious weed in the United States and is closely regulated. Dr J.D. Miller, USDA-ARS research leader of the Canal Point station, will be responsible for coordinating the collection's maintenance and activities.

A major environmental event that affected the World Germplasm Collection of Sugarcane and Related Grasses was hurricane Andrew. On 22 August 1992 the north eyewall of the storm, with sustained winds of over 165 mph, passed directly over the research station.

The loss of sugarcane germplasm is listed in Table 1. The *S. officinarum* clones were the most severely damaged group, with a loss of 46 per cent. These clones

were planted in the eastern most part of the field, which was saturated with salt water for many days after the storm. Fortunately, all 568 of these clones had been sent to Copersucar and were being maintained in Brazil. Replacement material has been received from the Copersucar collection and is currently in quarantine at Beltsville, Maryland, for re-establishment in the collection. The second important group to suffer significant loss was the *S. spontaneum*. These plants were maintained on trellis rows in pots due to their rhizomatous nature. Many of the pots were blown away and the others were blown over and their tags lost. Re-establishment of the *S. spontaneum* is currently in progress as many clones lost at Miami were in the breeding collection at Canal Point, Florida, and Houma, Louisiana.

The devastation caused in Miami by Hurricane Andrew points to the need for the back-up of sugarcane germplasm. Comparative inventories of the world collections need to be done periodically and non-duplicated clones need to be exchanged.

Sugarcane diseases detected and/or observed in the collection at Miami include smut, eye spot, rust, sugarcane bacilliform virus in *S. officinarum* clones, and symptoms of yellow leaf syndrome. Seedpieces cut from the smut infected clones were hot water treated at 52°C for 45 minutes. All plants propagated from seedpieces receiving this treatment were smut-free and retained. Plants in which either sugarcane bacilliform virus was detected or yellow leaf syndrome symptoms were observed were maintained as well as those with rust and eye spot. Systemic diseases, sugarcane mosaic and leaf scald, that were expected to be present have not been observed in the collection.

Besides the vegetatively maintained world germplasm collection, the USDA-ARS has financed and coordinated the preservation of true seed of both *S. officinarum* and *S. spontaneum* clones. Open pollinated true seed of 78 *S. officinarum* clones was produced at the Breeding Station of the Hawaiian Sugar Planters' Association and more than 100 seeds from each clone were stored in liquid nitrogen at the National Seed Storage Laboratory, Fort Collins, Colorado (Schnell et

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**Table 1.** Loss of sugarcane germplasm as a result of Hurricane Andrew.

Genus/species	June 1992	June 1995	Percentage lost
<i>Erianthus</i> spp.	87	87	0
<i>Miscanthus</i> spp.	33	18	45
<i>Miscanthus</i> hybrids	17	17	0
<i>Saccharum barberi</i>	55	55	0
<i>Saccharum edule</i>	29	10	66
<i>Saccharum</i> hybrids	202	196	3
<i>Saccharum officinarum</i>	568	305	46
<i>Saccharum robustum</i>	121	70	42
<i>Saccharum sinense</i>	37	37	0
<i>Saccharum spontaneum</i>	355	237	33
<b>Total</b>	<b>1 504</b>	<b>1 032</b>	<b>31</b>

al. 1994). True seed of 98 selfed *S. spontaneum* clones was produced at the Sugarcane Field Station in Canal Point and sent to the National Seed Storage Laboratory. The quantity of seed from the *S. spontaneum* clones was greater; 93 clones had in excess of 1000 viable seeds each and the remaining 5 clones had 500–900 viable seeds (Tai et al. 1994). Since the *S. spontaneum* project will continue for two additional years the number of clones with seed stored will increase.

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# Conservation of Sugarcane Germplasm in India Given the Occurrence of New Viral Diseases

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## Abstract

A world sugarcane germplasm collection is being maintained at the Sugarcane Breeding Institute Research Centre, Cannanore, India, in isolation from the commercial sugarcane belt. Recently the occurrence of the sugarcane bacilliform virus (SCBV) was noticed in noble cane genotypes (*Saccharum officinarum*), *S. barberi* and hybrids. Foliar symptoms of the virus disease were obvious in many clones. However, clones that were not exhibiting virus symptoms were positive to SCBV in ELISA tests. The presence of sugarcane mild mosaic virus (SCMMV) is suspected in clones of noble canes, *S. barberi* and hybrids. An unidentified disease with chlorotic spots and red mottle symptoms was noticed.

A WORLD GERmplasm COLLECTION is maintained at the Sugarcane Breeding Institute Research Centre, Cannanore, India. This station is 200 km from the commercial sugarcane belt. This location was selected to house the germplasm free of pest and disease, particularly mosaic disease which is common in all sugarcane tracts in India. The present number of clones in the world germplasm collection is 3345, comprising *Saccharum* spp., allied genera and hybrids (Table 1). The collection is replanted every year during the January–February planting season. The centre is looked after by a senior scientist (genetics and cytogenetics) with a pathologist and an entomologist making regular visits from Coimbatore to monitor for diseases and pests and to take necessary plant protection measures if required. In addition, 936 genotypes of *S. spontaneum*, *Erianthus* sp., *Narenga* sp., *Sclerostachya* sp., *Vetiveria* sp. and IND collections are maintained at the main campus, Coimbatore. Twenty-five clones of *Miscanthus* sp. and *Erianthus rufipillus*, which require a temperate climate, are maintained at Wellington, Nilgris, Tamil Nadu (Table 2).

Though occasional incidences of pests such as pyrailla and internode borer and diseases such as smut and rust have been reported, current management practices have effectively contained these. Recently the sugarcane bacilliform virus and the suspected sugarcane mild mosaic virus were recorded.

Smut has been recorded in a few clones. Genotypes of *Saccharum robustum* were found to be affected by the pathogen in some years. However, hot water treatment incorporating Bavistin (Carbendazim) or Bayleton (Tridimefon) was initiated to eliminate the pathogen before planting. This treatment has contained

**Table 1.** Genotypes in world germplasm collection maintained at Cannanore (Kerala).

Group/genotype	No. of accessions
1. <i>S. officinarum</i>	758
2. <i>S. barberi</i>	43
3. <i>S. sinense</i>	29
4. <i>S. robustum</i>	146
5. <i>S. spontaneum</i>	70
6. Allied genera	154
7. Indian collections	389
8. Indian hybrids	1 028
9. Foreign hybrids	578
10. IA clones	130
11. New accessions	20

**Table 2.** Germplasm accessions maintained at Coimbatore and Wellington.

Group/genotype	No. of accessions
<b>Coimbatore</b>	
<i>S. spontaneum</i>	654
<i>Erianthus</i> spp.	201
<i>Narenga</i>	25
<i>Sclerostachya</i>	
<i>Vetiveria</i>	
IND 90 collections	56
Total	936
<b>Wellington</b>	
<i>Miscanthus</i> sp.	25
<i>Erianthus rufipillus</i>	

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the pathogen. Technicians making daily visits to the field inspect for smut whips. Rust is not regularly noticed.

### Sugarcane Bacilliform Virus

The sugarcane bacilliform virus, a badna group virus closely related to the banana streak virus, was noticed in *S. officinarum* and some hybrid clones in 1992. Bacilliform virus particles were detected in genotypes Black Tanna and Listada (*S. officinarum*) and D1135 (hybrid), with suspected symptoms confirmed through electron microscopic and immunospecific electron microscopic studies (Viswanathan 1994).

Foliar symptoms (Figure 1) were chlorotic streaks of varying length, confined between veins. These foliar symptoms were apparent from first formed leaves in genotypes such as Black Tanna, Castilla, Listada and D1135. Symptoms of interveinal chlorosis were also seen in mature leaves of the infected genotypes Listada and D1135. Other symptoms in suspect genotypes were stunted growth in the severely infected noble cane genotypes of Black Tanna, Castilla, Guam A, and Listada and hybrid clones of D1135, D4/4 and D109. No tillering was noticed in these genotypes and poor tillering was noticed in many other noble canes with suspected SCBV infection. Another

interesting symptom in the severely stunted clones was deep longitudinal cracks on the internodal surface. The stunted canes showed progressive reduction in internodal elongation and at the apex leaves failed to open freely and formed a bunched top. Narrowing of the leaves was also noticed in some clones. Symptom expression varied between genotypes (Viswanathan, Alexander and Varma 1995). Expression of the clear foliar symptoms was seen in many *S. officinarum*, *S. barberi* and a few hybrid clones.

Direct antigen coating enzyme linked immunosorbent assay (DAC-ELISA) was standardised to detect virus infection in sugarcane clones using serum produced against SCBV (supplied by Dr B.E.L. Lockhart). ELISA tests showed that many genotypes without foliar symptoms of the virus tested positive to the virus. Studies showed that 49 out of 125 clones of noble canes and 21 out of 33 hybrid clones were positive to the virus (Viswanathan and Alexander 1994). At 6–8 months of age many of the severely affected hybrid clones, including D1135, D1135 Str, D908, D109, D4/4 and D14/34, and noble canes such as Black Tanna, Listada, Boetata, Bilatote, Local Pattapatti, Gros Genoux and Castilla were showing drying in the field. One set of these genotypes is now maintained in pots in the glasshouse and constantly monitored.

### Sugarcane Mild Mosaic Virus

The presence of this virus was suspected in some of the clones in 1994. Symptoms of this virus were intense mottling throughout the laminar region and mild mosaic. However, the clostero virus was not associated as reported in other countries. Symptoms of stunted growth, internodal splitting and drying were not observed in the clones with suspected SCMMV infection. However, many genotypes showed symptoms of both SCBV and SCMMV.

### Problems Facing the Indian Sugarcane World Germplasm Collection

There are serious concerns about the disease status in the germplasm collection following the presence of SCBV and SCMMV in noble canes and other clones at Cannanore. SCBV symptoms are expressed in many of the noble canes and some clones of *S. barberi*. Imported hybrids and Indian hybrids have no conspicuous symptoms. DAC-ELISA studies showed that C279, CP31-588, CP47-193, F31-436, F31-407, F31-762, F32-8560, Q69 and POJ2725 (all hybrid clones) were positive to SCBV but none of them showed symptoms either at Cannanore or at Coimbatore (Viswanathan and Alexander 1994). Though SCBV infection was reported to be higher in noble canes than in commercial hybrids (Comstock and Lockhart 1990) all hybrids more than 50 years old and four of 21 current varieties



Figure 1. Foliar symptoms in genotype D1135.



were infected by SCBV in Australia (Teakle and Egan 1994). The various heat treatments used for controlling sugarcane diseases failed to free infected cuttings from SCBV (Autrey et al. 1990). Though SCBV infection was not reported in previous studies to reduce yields, at Cannanore many genotypes showed reduced growth and premature drying (Viswanathan et al. 1995).

The association of SCMMV with SCBV may add to the problems in the Indian collection. Although SCMMV has not been confirmed, its presence was suspected last year and many noble canes showed typical symptoms of SCMMV. The spread of the SCBV virus to disease-free clones was suspected since many clones that were not showing symptoms have expressed symptoms recently. Since both SCBV and SCMMV were reported to occur together (Lockhart, Autrey and Comstock 1992) the combined effect of the two viruses on sugarcane and its yield will be studied.

### Prospects

Maintenance of the germplasm collection is meticulously planned and the replanting of more than 3000 genotypes every year involves large resources. Routine quarantine measures are conducted by plant protection scientists. Although there have been difficulties in maintaining some noble canes and hybrid clones infected by SCBV no germplasm has been lost. Hot water treatment, combined with meristem culture, has been routinely practised to free Indian hybrids of the sugarcane mosaic virus to include them in the germplasm collection. Future studies will be on eliminating viruses and other diseases through meristem culture and other methods. Cryopreservation is a technique that may serve as an additional security for accessions. In sugarcane no decrease in viability occurred following precooling to  $-100^{\circ}\text{C}$  and storage in liquid nitrogen for four months (Chen, Gavinlertvatana and Li 1979). However, all stages in the cryopreservation procedures — namely, pregrowth, cryoprotection freezing, storage time, thawing and recovery — play a crucial role in attaining success. Srinivasan and Srinivasan (1988) reported that different species of *Saccharum* responded differently to the tis-

sue culture conditions; *S. robustum* and *S. spontaneum* were more difficult to establish than *S. officinarum*, *S. sinense* and *S. barberi*. Improved molecular characterisation of the viruses, the development of molecular diagnostic test protocols and the establishment of epidemiological aspects of the diseases would assist with identifying diseased genotypes and would enable plans for plant protection/phytosanitary practices to be developed.

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**Major Diseases Affecting  
Sugarcane Production and  
Recent Experiences with  
Sugarcane Diseases in  
Quarantine**

# Major Diseases Affecting Sugarcane Production in the United States

J.C. Comstock\*

## Abstract

The major diseases affecting sugarcane production in the United States are ratoon stunt, rust, leaf scald, mosaics, smut and yellow leaf syndrome. Scientists are developing techniques for detecting pathogens of the major diseases, which should ensure safer quarantines and more effective control practices.

DISEASES affect commercial sugarcane production in the United States of America wherever the crop is grown. The major diseases are listed by location in Table 1. As well as the direct yield losses, diseases have a major impact on cultivar development programs, causing susceptible cultivars to be discarded and restricting the use of certain cultivars as parents. Another economic impact is the requirement of costly disease control and management practices to prevent yield losses. Hot water treatment of seedcane, maintenance of disease-free nurseries, and inspection programs all increase the cost of producing sugarcane.

Ratoon stunting disease, caused by *Clavibacter xyli* ssp. *xyli*, is found in all sugarcane production areas and probably causes the most yield loss of any disease in the United States. A high incidence of ratoon stunting disease has been reported in Louisiana where heat treatments and sanitation are routinely practised (Damann and Hollier 1991) and nearly 100% stalk incidence occurred in Florida in certain cultivars without these control practices. Yield losses of 5% industry-wide have been estimated in Florida based on losses in yield tests and the commercial production of these cultivars (Dean and Davis 1990). Besides the direct yield losses, indirect costs in disease control practices affect growers in all industries. Hot water treatment of sugarcane setts for seedcane nurseries is expensive.

Common rust, caused by *Puccinia melanocephala*, is important in Florida and Hawaii. In Florida its incidence varies depending on weather conditions, year, cultivar and field location. A lack of a winter freeze and cool spring temperatures favour rust epidemics (Irey 1987) that decreased sugar yields of cultivar CP78-1247 by 39.3% during 1988-89, based on historical yields (Raid, Anderson and Coale 1989). Varieties have been withdrawn from commercial production because of rust susceptibility in Hawaii and Florida and have been discarded because of susceptibility in the cultivar development programs. The

harsher winters in Texas and Louisiana appear to decrease rust inocula and prevent major epidemics.

Historically sugarcane mosaic has been important in the Louisiana sugar industry, and was a problem after the industry was established in Texas. High levels of mosaic are still found in some of the older commercial cultivars in Louisiana. Sugarcane mosaic has been occasionally observed in Florida. Commercial cultivars have been withdrawn from production in Louisiana and Florida. In Hawaii, mosaic has been seldom seen. All mainland sugarcane development programs screen cultivars for resistance, discarding susceptible cultivars.

Smut had a major impact on each area of the US sugarcane industry after its introduction. Commercial cultivars were withdrawn because of smut susceptibility in every location. In Hawaii in the late 1970s, yield losses of 25% occurred in certain fields of H50-7209, a cultivar susceptible to smut race B, based on historical and subsequent yields. At present, resistant cultivars are grown in all locations.

Leaf scald increased in incidence in Florida starting in 1989 from an almost near zero incidence and the disease has subsequently been observed in Texas and Louisiana. In Florida, serological techniques detected *Xanthomonas albilineans* in ten commercial fields of CP80-1743, ranging from 1.1% to 12.2% stalk infection in 1994. The new strain of *X. albilineans* that was reported by Davis (1992) in Florida is believed to be

**Table 1.** Major diseases in the United States of America affecting sugarcane production, by State<sup>a</sup>.

Disease	Florida	Hawaii	Louisiana	Texas
Ratoon stunt	X	X	X	X
Rust	X	X		
Leaf scald	X	X	X	
Mosaic			X	X
Smut		X	X	X
Yellow leaf syndrome	?	?	?	?

<sup>a</sup> X indicates disease is present.

\*USDA-ARS, Sugarcane Field Station, Star Route Box 8, Canal Point, Florida 33438, USA.

**Table 2.** 'New' sugarcane diseases detected in the United States of America, by State.

Disease	Florida	Hawaii	Louisiana	Texas
Dry top rot	1991			
Leaf scald	1989 <sup>a</sup>		1992	199? <sup>b</sup>
Red leaf mottle	? <sup>c</sup>			
Purple spot	1993			
Sugarcane bacilliform virus	1990	199? <sup>b</sup>	199? <sup>b</sup>	199? <sup>b</sup>
Sugarcane mild mosaic virus	1992			
Yellow leaf syndrome	1993	1989	199? <sup>b</sup>	199? <sup>b</sup>
Yellow spot	1985			

<sup>a</sup> New strain. <sup>b</sup> Unable to obtain exact date of discovery. <sup>c</sup> Presence questionable; symptoms and peanut clump virus-like particles found in CP cultivars shipped to Mauritius.

**Table 3.** Scientists developing techniques for detecting sugarcane disease pathogens in the United States of America.

Pathogen	
<b>Bacterial pathogens</b>	
	<b>Polymerase chain reaction</b>
<i>Clavibacter xyli</i> ssp. <i>xyli</i> (ratoon stunting)	
K. Damann	LSU, Baton Rouge, La.
M.J. Davis	UF, IFAS, TREC, Homestead, Fl.
<i>Xanthomonas albilineans</i> (leaf scald)	
Y.B. Pan, D. Burner, M. Grisham	USDA, ARS, Houma, La.
K. Damann	LSU, Baton Rouge, La.
M.J. Davis, P. Rott, D.W. Gabriel	UF, IFAS, TREC, Homestead, Fl.
R. Honeycutt	Calif. Inst. Bio. Res., LaJolla, Ca.
N. Schaad	USDA, ARS, Frederick, Md.
<i>X. campestris</i> pv. <i>vasculorum</i> (gumming disease)	
R. Honeycutt	Calif. Inst. Bio. Res., LaJolla, Ca.
N. Schaad	USDA, ARS, Frederick, Md.
<i>Pseudomonas rubrilineans</i> (red stripes)	
R. Honeycutt	Calif. Inst. Bio. Res., LaJolla, Ca.
<i>P. rubrisubalbicans</i> (mottle stripe)	
R. Honeycutt	Calif. Inst. Bio. Res., LaJolla, Ca.
	<b>Monoclonal antibodies (Sero var specific)</b>
<i>X. albilineans</i> (leaf scald)	
A. Alvarez	UH, Honolulu, Hi.
<b>Fungal pathogens</b>	
	<b>Polymerase chain reaction</b>
<i>Ustilago scitaminea</i> (smut)	
S. Schenck	HSPA, Aiea, Hi.
<i>Pythium arrhenomanes</i>	
J. Hoy	LSU, Baton Rouge, La.
<b>Viral pathogens</b>	
	<b>Polymerase chain reaction</b>
Sugarcane bacilliform virus	
B. Lockhart	UM, St. Paul, Mn.
Sugarcane mild mosaic virus	
T.E. Merkof	Texas A&M Univ., Weslaco, Tx.
Yellow leaf syndrome	
B. Lockhart	UM, St. Paul, Mn.
M.S. Irely	U.S. Sugar Corp., Clewiston, Fl.

responsible. In Louisiana, leaf scald was first detected in 1992. The levels of symptomatic leaf scald plants during the early part of the season necessitated the destruction of one field of CP74-383. Stalk sucrose content was lower in symptomatic compared with asymptomatic stalks (Hoy and Grisham 1994). Unfortunately some newly released cultivars, CP74-383 and LCP82-89, in Louisiana are susceptible and the future impact of the disease is unknown. Both cultivar development programs in Florida and Louisiana have been affected with the loss of susceptible clones.

Yellow leaf syndrome caused yield losses that resulted in the withdrawal of H65-7052 in Hawaii. Yellow leaf syndrome is known to occur in both Florida and Texas in commercial fields. The yield of one commercial cultivar, CP72-1210, with yellow leaf syndrome has decreased over the years in Florida. Whether it is due to only yellow leaf syndrome is not known.

A number of 'new' diseases were detected in certain areas of the United States during the late 1980s and early 1990s. These diseases include dry top rot, leaf scald, purple spot, sugarcane bacilliform virus, sugarcane mild mosaic virus, yellow leaf syndrome, and yellow spot (Table 2). The concern about leaf scald is because its incidence in both Florida and Louisiana is increasing.

Detection techniques for pathogens of the major diseases and the 'new' diseases are currently being developed in the United States by various scientists (Table 3). Once these detection techniques are in place they will ensure safer quarantines and enable research to develop resistant cultivars and more effective control practices.

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# Acquisition and Quarantine of *Saccharum* spp. Germplasm and Related Genera in the United States

S.S. Hurtt\*

## Abstract

In the United States the quarantine and indexing of sugarcane germplasm is entrusted to the quarantine unit of the US Department of Agriculture, Agricultural Research Service, National Germplasm Resources Laboratory. Imported germplasm is held in quarantine for 18–24 months. During this time, clones are subjected to a short and a long hot water treatment to eliminate pest and pathogens potentially present in the plants or plant parts. The propagants are observed for disease symptoms during each of the two growth cycles following the hot water treatments. Diagnostic tools applied for pathogen detection include tissue imprinting and immunoassays for leaf scald and ratoon stunt causing bacteria, culture on semi-selective medium for leaf scald bacterium, and bioassays and leaf dip electron microscopy for virus detection. In the past five years, about 455 acquisitions have entered the quarantine from 14 different geographic areas. Less than 5% were retained due to the presence of pathogens. Mosaics and ratoon stunt are the most frequently encountered diseases.

QUARANTINE AND INDEXING of *Saccharum* spp. and germplasm of related genera are performed in the United States by the quarantine unit of the National Germplasm Resources Laboratory (NGRL). NGRL is one of many US Department of Agriculture, Agricultural Research Service laboratories located at the Beltsville Agricultural Research Center in Maryland. The NGRL quarantine unit is responsible for the quarantine and indexing of more than 50 plant species. However, the majority of the unit's resources are used in the quarantine of pome and stone fruits, sugarcane and related genera, potatoes, sweet potatoes, rice, small fruits and brambles, and grasses.

## Organisational and Resources Data

Sugarcane is unique among genera processed by NGRL because the laboratory provides the quarantine for the interstate, as well as the international, movement of germplasm. The primary participants in the interstate exchange program are Florida, Louisiana, Texas and Maryland. Because of the disease status in Hawaii, germplasm brought to mainland United States from this state must be treated as foreign germplasm. Germplasm from the mainland is isolated, observed and indexed over a 6–8 month period. Foreign germplasm, on the other hand, is held in quarantine for 18–24 months. This paper elaborates on the quarantine for foreign germplasm exchange.

The facilities dedicated to sugarcane quarantine include four greenhouses with 300 m<sup>2</sup> of space, a head-house area, and a small laboratory for doing hot water treatments and preparing shipments. An additional office and laboratory provide space for maintenance of records, computer hardware, and equipment for diagnostic testing. The author supervises the sugarcane program, as well as the programs for potatoes, sweet potatoes and pome fruits.

## Germplasm Acquisition and Tracking

Upon entering the United States, sugarcane is inspected by US Department of Agriculture Animal and Plant Health Inspection Service (APHIS) personnel. If no signs or symptoms of pests or pathogens are detected, the propagation materials (usually cane setts or stools) are passed on to the NGRL quarantine unit staff. NGRL logs in the new accessions by assigning each an identification number — that is, 'Qnumber'. Pertinent data including geographic origin, genus and species, clone designation, date of arrival, and designated recipient(s), are entered into the NGRL quarantine unit computer database and electronically exported to the national database known as GRIN (Germplasm Resources Information Number). The database is accessible worldwide by modem. Contact Mr Jim Mowder, head of the NGRL database management unit, for information (facsimile: 301-504-6305).

Since 1990, about 455 accessions have been imported through the NGRL quarantine unit. The sources of these accessions are shown in Table 1. The yearly acquisitions vary from 13 (1993) to 165 (1994). The activity in 1994 was due largely to the acquisition of germplasm from the Brazilian collection to replace

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clones destroyed in Florida by hurricane Andrew. In addition to acquisitions listed in Table 1, 75–100 plants were processed annually in the interstate exchange of germplasm. The primary domestic recipients of released germplasm, in order of number of accessions received, are Florida, Hawaii, Texas, Louisiana and Maryland.

### The Quarantine Process

When the NGRL quarantine unit receives cane setts or stool pieces, they are given a short hot water treatment (52°C for 30 minutes). The cane pieces or stools are then germinated and grown on raised benches in a greenhouse dedicated to new acquisitions. The first new growth is sampled for bioassay on *Sorghum bicolor* 'Rio' to detect sugarcane mosaic-like viral pathogens. The sugarcane plants are observed for 6–9 months for signs or symptoms of pests or pathogens. If the sugarcane matures without symptoms of disease, the stalks are cut and the basal segments are used in serological and isolation tests for pathogens. Tissue imprints are made onto nylon membranes using low speed centrifugation. The membranes are then tested with antisera to *Xanthomonas albilineans* or *Clavibacter xyli* ssp. *xyli* to diagnose leaf scald or ratoon stunt diseases (Comstock and Irey 1992). In addition, stalk pieces are pressed onto a semi-selective medium for the isolation of *X. albilineans* (Davis et al. 1994).

The remainder of the stalk is soaked in cold water for 72 hours, then treated in hot water at 50°C for 2 hours (long hot water treatment). Treated setts and stools are germinated and grown in a greenhouse

separated from that used to grow the short hot water treated plants. The plants are observed for symptoms as they mature, recommended for release if symptomless, then cut for distribution. At the time of harvest, cane pieces are again tested serologically and on semi-selective mediums for ratoon stunt and leaf scald bacteria.

If any plant develops symptoms during either of the two growth cycles, additional tests are undertaken to determine a probable cause. For example, leaf pieces with viral-like symptoms are examined by leaf dip electron microscopy for viruses. Under current laboratory policy, the detected viruses are not characterised to determine their taxonomic or strain classification. When suspicious symptoms persist in a plant after the long hot water treatment and no viral pathogen is detected by electron microscopy or bioassay, the plant is considered to be infected by an unidentified pathogen(s). Since the unit currently has no program for eliminating viruses from *Saccharum* spp. and related genera, accessions with virus-like symptoms are destroyed and sources of healthy replacement material are sought.

### Diseases Intercepted

Less than 5% of the acquisitions are destroyed because they harbour pathogens. The diseases most frequently intercepted are those with mosaic-like symptoms and ratoon stunt. Plants with maize streak virus-like symptoms are occasionally encountered and withheld. One plant has been diagnosed with leaf scald by using serological and isolation tests for the bacterium. Detection of sugarcane badnavirus has

**Table 1.** Number of accessions acquired from different geographic areas since 1990 by the US Department of Agriculture NGRL sugarcane quarantine unit.

Geographic source of imports	Acquisitions by year					Total by origin
	1990	1991	1992	1993	1994	
Argentina	4	0	0	0	0	4
Australia	22	0	20	0	0	42
Brazil	14	2	1	0	140	157
China	0	7	30	6	0	43
Colombia	0	13	9	0	0	22
Dominican Republic	0	12	0	0	0	12
Fiji	29	0	0	0	0	29
Hawaii	6	21	1	0	3	31
India	71	0	0	0	4	75
Mauritius	0	0	0	0	14	14
Mexico	0	6	0	0	0	6
Pakistan	0	0	0	0	3	3
South Africa	0	2	0	0	1	3
Taiwan	6	0	1	7	0	14
<b>Total by year</b>	<b>152</b>	<b>63</b>	<b>62</b>	<b>13</b>	<b>165</b>	<b>455</b>

been discussed by representatives of the Agricultural Research Service and the Animal and Plant Health Inspection Service. It was decided that, since the virus is distributed widely in the noble canes, NGRL should not test for this virus.

### Future Directions

The Agricultural Research Service and the Animal and Plant Health Inspection Service are jointly moving forward with the construction of new greenhouse and handling facilities for the sugarcane quarantine program. Requirements have been delivered to the architects and engineers for design. Construction is planned for 1997.

NGRL is interested in applying new technologies to improve efficiency in detecting pathogens. Current interests are in developing ELISA assays for potyvirus and maize streak virus detection. It has been found that isolates of the sugarcane mosaic virus strain F from Pakistan did not react with a broad spectrum monoclonal antibody for potyviruses. The isolates were sent to Dr S. Jensen, Agricultural Research Service,

Lincoln, Nebraska. He found that the biochemical properties of the virus are not characteristic of a potyvirus (Jensen and Hall 1993). He has tentatively renamed the virus sorghum necrotic mottle virus. The NGRL quarantine unit is continuing to collaborate with Dr Jensen on characterisation of the virus. It is collaborating also with Dr R.E. Davis, Agricultural Research Service, Beltsville, MD, to develop nested-primer PCR technology for detecting phytoplasmas. Lastly, it is evaluating the use of bioassays on *Chenopodium* spp. for detecting peanut clump virus (red leaf mottle).

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# Major Diseases Affecting Sugarcane Production on the Ramu Sugar Estate, Papua New Guinea

S. Suma and E. Pais\*

## Abstract

The major diseases affecting sugarcane production on the Ramu sugar estate in Papua New Guinea are downy mildew *Peronosclerospora sacchari* (T. Miyake) Shirai & K. Hara, and Ramu stunt. These diseases are responsible for the discarding of 35% of all imported varieties. Ramu orange leaf, Ramu scorch, Ramu streak, rusts (*Puccinia melanocephala* H. Syd & D. Syd and *P. kuehnii* Butl.), leaf scald *Xanthomonas albilineans* (Ashby) Dowson, and various other diseases are known to occur on the estate but have minimal effect on sugarcane production.

NEW GUINEA is generally accepted as a centre of diversity for the genus *Saccharum* (Daniels and Roach 1987), with several species native to Papua New Guinea. Numerous clones of the noble cane, *S. officinarum*, are grown as individual stools in subsistence gardens. *S. edule* is grown for its aborted inflorescence, which is used as a vegetable, and *S. spontaneum* and *S. robustum* occur naturally in the grasslands and on river banks.

This reservoir of diverse genetic material has great potential for the sugarcane industry and has received the attention of many collecting expeditions in the past for variety improvement programs (Krishnamurthi and Koike 1982). As it is a centre of diversity, many pests and diseases of sugarcane would have co-evolved with the crop in Papua New Guinea, and pressure from these events on commercial production would be quite severe. This proved partly true with the Ramu stunt epidemic of 1985 (Eastwood 1990; Egan et al. 1989) and the outbreak of pests such as *Sesamia griseocens* Walker (Lep. Noctuidiae) (Kuniata and Sweet 1991). Commercial sugarcane production in Papua New Guinea was begun in 1979 by Ramu Sugar Ltd on a nuclear estate located in the Ramu valley, approximately 5° 50'S and 145° 40'E, 400 m above sea level. The annual rainfall is about 1950 mm and most of it occurs between late September and early June as a consequence of the north-west monsoon.

## Fungal Diseases

### Downy mildew

Downy mildew (*Peronosclerospora sacchari* [T. Miyake] Shirai & K. Hara) is one of the most important diseases on the Ramu estate, with cane yield losses ranging from 2% to 15%. Disease symptoms

where affected plants show pale to yellow coloured leaves and thin slender stalks were summarised by Leu and Egan (1989). Being systemic, infected planting material is the primary source of disease spread. Yield loss studies conducted between 1988 and 1990 estimated that a 1% infected stalk level resulted in a yield loss of 0.22 tonnes of cane per hectare. Several varieties with high sugar yields are not cultivated because they are highly susceptible to downy mildew, and this can also be considered a loss of production. More than 36% of all imported varieties tested since 1979 were discarded due to downy mildew (Table 1).

Downy mildew is controlled on the estate primarily by using tolerant varieties. Cultivars rated 5 or less on the International Society of Sugar Cane Technologist's 1 to 9 scale of resistance are considered for commercial production. Most of the introduced varieties came via the Bureau of Sugar Experiment Stations quarantine in Brisbane (Queensland, Australia) and screening for downy mildew resistance is done by Ramu Sugar Ltd. The varieties that consistently give a 5 or lower rating after two consecutive downy mildew screening trials are selected for further agronomic evaluation. Metalaxyl (Ridomil Plus 70, Ciba-Geigy AG) at 1.25 g a.i./L as a pre-plant treatment (James 1983; Malein 1993) has been shown to reduce downy mildew levels in planting setts. The importance of developing 'clean' seedcane nurseries has been realised and this will be attempted in future plantings.

In 1988, variety Q107 and Cassius, which were relatively tolerant to downy mildew in trials off the estate, were showing unacceptably high levels of downy mildew. It was later realised that a strain change may have evolved and this was named as downy mildew strain B. This meant that any control strategy for downy mildew at the estate may have to be flexible to accommodate such shifts in disease pathogenicity.

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## Ramu orange leaf

Ramu orange leaf disease was first recorded in the late 1980s in a commercial crop of Cassius and a propagation plot of Q132. It was not until 1993 that large numbers of infected stools were observed in commercial crops of RQ117, Q127 and BT65152. Ramu orange leaf is caused by a fungal organism, tentatively identified by the International Mycological Institute as belonging to the order *Exobasidiales*. The nature of the disease is largely unknown, except that disease levels of up to 920 stools/ha (range: 535–1156) or 3800 stalks/ha (range: 2234–5741) have been observed in commercial crops of RQ117. The disease level in the field increases in the first three months after harvesting, then drops thereafter with the progressive death of infected shoots. The disease has been observed largely in ratoon crops and is not a problem in plant cane. No yield loss estimates have been made. However, it is currently considered minor. No control strategy has been adopted but disease levels in commercial fields

are routinely monitored as part of the estate's disease survey program.

## Rust

Both common rust (*Puccinia melanocephala* H. Syd & P. Syd) and orange rust (*P. kuehnii* Butl.) occur on the estate in very low levels and in certain varieties only. Common rust had not been recorded in the country until 1991 when it was found infecting crops of variety BT65152 at the Ramu estate. However, its effect on cane yield is minimal and the use of this variety is currently being expanded in commercial plantings.

## Minor fungal diseases

Other fungal diseases that occur on the estate but have an insignificant effect on sugarcane production include leaf splitting disease (*P. miscanthi* [Miyake] C.G. Shaw), zonate leaf spot (*Gloeocercospora sorghii* D. Bain & Edg.), pokkah boeng (*Fusarium moniliforme* Sheld.), wilt (*Cephalosporium sacchari* Butl.), red rot

**Table 1.** Sugarcane varieties of the world tested for downy mildew and Ramu stunt resistance at Ramu between 1979 and 1992.

Country of origin	Total number of varieties tested	Number of downy mildew tolerant varieties	Percentage selected for downy mildew tolerance	Total number of varieties tested	Number of Ramu stunt tolerant varieties	Percentage selected for Ramu stunt tolerance	Current commercial varieties by country of origin
Argentina	12	9	75.0	5	5	100.0	0
Brazil	44	24	54.5	22	21	95.5	0
CSR, Australia	19	12	63.2	20	10	50.0	1
Cuba	10	10	100.0	3	3	100.0	0
Fiji	4	3	75.0	4	1	25.0	0
Hawaii	39	23	59.0	35	32	91.4	1
India	35	23	65.7	20	15	75.0	1
Japan	10	8	80.0	0	0	na	0
Java/Indonesia	4	3	75.0	1	0	0.0	0
Kenya	3	1	33.3	2	1	50.0	0
Mauritius	16	13	81.3	3	1	33.3	0
Mexico	1	1	100.0	0	0	na	0
Philippines	5	3	60.0	5	4	80.0	0
Puerto Rico	1	1	100.0	1	1	100.0	0
Queensland	101	54	53.5	55	37	67.3	4
Reunion	12	8	66.7	2	1	50.0	0
Saipan	1	1	100.0	1	1	100.0	0
South Africa	8	6	75.0	5	4	80.0	0
Taiwan	20	18	90.0	16	12	75.0	0
USA	46	32	69.6	11	5	45.5	0
West Indies	44	27	61.4	37	23	62.2	2
Total	435	280		248	177		9
Percentage of number tested		64.4			71.4		

na Not available.

(*Glomerella tucumanensis* [Speg] van Arx & E. Muller), yellow spot (*Mycovellosiella koepkei* [Krager] Deighton), veneer blotch (*Deightonella papuana* Shaw), ring spot (*Leptosphaeria sacchari* B. de Haan), red leaf spot (*Dimeriella sacchari* [B. de Haan] Hansford), black leaf spot (*Phyllachora sacchari* P. Henn) and brown spot (*Cercospora longipes* Butl.).

## Bacterial Diseases

### Leaf scald

Leaf scald (*Xanthomonas albilineans* [Ashby] Dowson) was first confirmed on the estate in 1988. The disease was probably introduced into the country with imported varieties. Variety Cassius was phased out from commercial planting as a result of leaf scald and strain B of downy mildew. Symptoms have not been observed in current commercial cane fields. However, it continues to occur in low levels in susceptible varieties in the museum (germplasm conservation) and the propagation plots. The main control regime adopted against leaf scald is the use of tolerant varieties, and resistance screening is done together with other diseases in downy mildew screening trials.

### Ratoon stunting disease

Ratoon stunting disease is caused by the bacterium *Clavibacter xyli* ssp. *xyli* Davis, Gillaspie, Vidaver & Harris, which causes stunting and unthrifty growth of cane (Gillaspie and Teakle 1989). However, disease symptoms can be confused with stress factors such as poor nutrition and dry conditions. Several ratoon stunting disease surveys have been conducted by Ramu Sugar Ltd but no ratoon stunting disease was detected on the estate. However, this observation may not necessarily be true for Papua New Guinea as a whole.

## Viral Diseases

### Ramu stunt

Ramu stunt was first confirmed infecting cane on the estate in 1985 (Egan 1986; Waller, Egan and Eastwood 1987). Symptoms are characterised by severe stunting, excessive reduction in stool size and a number of diseased stools showing a 'grassy' appearance. The causal organism is thought to be a virus (Jones, Antoniw and Eastwood 1989). However, this has not been confirmed. Kuniata et al. (1994) have shown that the causal organism is transmitted by a leaf hopper, *Eumetopina flavipes* Muir (Hom. Delphacidae). There was up to a 40% reduction in total sugarcane production from the 1986 crop (Eastwood 1990). The crop was largely of the variety Ragnar, which is quite susceptible to Ramu stunt. The drop in sugarcane yield was from a four year average of 70.75 t/ha to 26 t/ha

(Eastwood 1990). Up to 30% of all imported varieties tested were shown to be susceptible to Ramu stunt (Table 1). In an attempt to minimise losses, all imported varieties are screened for their susceptibility to Ramu stunt and those that are rated as resistant are selected for further agronomic evaluation. In the future, varieties that have intermediate reactions but acceptable cane and sugar yield will be evaluated for commercial exploitation by Ramu Sugar Ltd.

### Fiji disease

Fiji disease was one of the main concerns at the inception of Ramu Sugar Ltd. However, this has not proved to be the case long term. Some Fiji diseased canes were observed in the 1980s in the commercial fields, but in the past 5–7 years, the disease has not been observed in commercial cane. Although it can significantly reduce sugarcane yields in other sugarcane growing regions (Egan et al. 1989), it has no significant effect on sugarcane production on the estate. Fiji disease is one of the diseases considered in screening trials, and susceptible varieties are not promoted to commercial scale plantings.

## Unknown Diseases

### Ramu scorch

Ramu scorch was described by Egan (1986) as producing straw coloured spots with red-brown margins and a yellowish halo — somewhat similar to 'leaf scorch' except in the very early stages of disease development. Its association with the Ramu stunt epidemic in 1986 was thought to have an impact on cane yield. However, currently it is considered to be minor. The causal organism has not been identified but *Lophops* spp. has been implicated as the vector of the disease.

### Ramu streak

Ramu streak symptoms are somewhat similar to chlorotic streak. However, unlike chlorotic streak, the disease rarely causes leaf necrosis, has a more yellow-green colouring, and does not produce the characteristic broken irregular streaks parallel to the leaf venation. The disease was first seen in the mid-1980s but gained prominence only in early 1994 with widespread and extensive symptoms in crops of Q127, RQ117 and BT65152. Possible yield losses have not been determined.

## Conclusions

In the 'home' of sugarcane there may still be a lot of unknown diseases and pests in the wild that could become important in the future. Downy mildew and Ramu stunt remain the most important diseases on the Ramu estate and together were responsible for the discarding of 35% of all imported varieties between 1979

and 1990. The use of resistant or tolerant varieties is the key to reducing disease incidence and yield losses on the estate. Metalaxyl pre-plant treatments for controlling downy mildew can be quite effective and this should be done at primary nurseries. The other diseases including Ramu orange leaf, leaf scald, Fiji, Ramu scorch, Ramu streak and rusts currently have only a minor effect on yield. However, the potential threats from these diseases are recognised.

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# The Sugarcane Disease and Quarantine Situation in Southern Africa

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## Abstract

Although a large number of sugarcane pathogens have been recorded from southern Africa, the current disease situation in commercial crops is relatively simple. The various countries can be regarded as one phytosanitary area as far as sugarcane is concerned. Diseases of current or potential commercial importance are mainly ratoon stunting disease, smut, mosaic, leaf scald, rust and red rot (the latter following borer damage).

SUGARCANE is grown under rainfed conditions in the southern part of the South African cane belt. Here rainfall is low (long term mean is about 1000 mm a year) and erratic, and mean temperatures are relatively cool. In the northern parts of the South African industry and in neighbouring countries the crop is grown under full irrigation in semi-arid climates (long term mean rainfall is as low as 500 mm a year). Here summer temperatures are warm but winters are cool. Rainfall is markedly seasonal throughout the entire southern African cane belt and falls predominantly in summer.

The varieties grown in the different countries originate mainly from the South African Sugar Association Experiment Station (SASEX) breeding program. (Zimbabwe has its own local selection program based on fuzz from SASEX parent varieties at Mount Edgecombe.) Although there has been a marked shift from NCo to N varieties in South Africa and Malawi during the past 15 years, NCo376 is still the dominant variety in Swaziland, Zimbabwe and Zambia. NCo376 is highly susceptible to smut and mosaic, although conditions are not favourable for mosaic where this variety is still widely grown.

## Diseases

In terms of economic significance, ratoon stunting disease is by far the most important disease in the entire sub-continent, being common and often very severe in some industries. Smut is common wherever NCo376 is grown. In estate-grown cane in Swaziland, Zimbabwe and northern Malawi smut is maintained at stable low levels in NCo376 by intensive roguing, but elsewhere smut is often very serious in this variety. Smut-susceptible and mosaic-susceptible varieties are

no longer grown in those parts of the South African industry where these diseases were formerly serious problems.

Leaf scald is endemic in the northern part of the South African industry and in all other countries but all varieties grown are resistant. Conditions are generally favourable for rust but again all varieties grown have good resistance.

New genotypes in the SASEX selection program are intensively screened or selected for resistance to smut, mosaic, leaf scald and rust. Some selection for resistance to gumming and various leaf spotting diseases is also practised. Advanced genotypes are tested for resistance to streak and to red rot.

Sugarcane bacilliform virus and mild mosaic virus have been recorded in South Africa and Malawi. Both probably occur in other industries in the sub-continent.

**Table 1.** Imports of sugarcane varieties into South Africa from 1984 to 1994.

Argentina	9
Australia	20
Brazil	12
Colombia	6
Japan	15
Mauritius	17
Mexico	8
Réunion	10
Taiwan	14
USA - Florida	28
- Louisiana	7
- Hawaii	9
Zimbabwe	109
<b>Total</b>	<b>264</b>
	<b>(26 destroyed)</b>

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### **Imports**

The recommendations of the International Board of Plant Genetic Resources, the Food and Agriculture Organisation of the United Nations and the International Society of Sugar Cane Technologists are generally followed for imports of sugarcane varieties. There is no routine indexing for diseases except for ratoon stunting disease and leaf scald by immunofluorescence microscopy.

The diseases that have been intercepted in quarantine are smut, leaf scald and ratoon stunting disease.

### **Exports of varieties from South Africa**

All materials are obtained from hot water treatment nurseries on SASEX field stations and are carefully inspected for symptoms of systemic pathogens. No indexing for diseases is practised, except microscopic checking for ratoon stunting disease. There is a slight to very slight risk of smut, leaf scald, mosaic virus and sugarcane bacilliform virus, depending on the station of origin.

# Problems of Pests and Diseases for Sugarcane and the Resistance Breeding Program in Indonesia

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## Abstract

Top and stem borer, woolly aphid and scale are present in most sugarcane areas in Indonesia. Other pests, although important, are specific to certain areas. The disease resistance breeding program routinely conducted against mosaic, leaf scald and pokkah boeng diseases has decreased the level of importance of these traditional sugarcane diseases in Indonesia. However, other diseases such as smut and ratoon stunting are becoming important because they are present in most sugarcane plantations in Indonesia.

INDONESIA, especially its eastern province Irian Jaya, is believed to be a part of the centre of origin for sugarcane. Together with New Guinea, Irian Jaya has frequently been explored to find and save sugarcane germplasm. The sugarcane germplasm collection of the Indonesian Sugar Research Institute is maintained in Pasuruan, east Java, and consists of about 761 clones comprising six *Saccharum* species. Most of them are *S. officinarum*. The Institute also maintains another 4500 hybrids (Lamadji 1994a). These collections are maintained in the field and replanted every year. To broaden the genetic base, the Institute has also introduced some clones from overseas from, for example, world collections and institutions such as BSES Australia, TSRI Taiwan and SBI Coimbatore.

The sugar industry in Indonesia is spread across the Medan area in north Sumatra, south Sumatra, Java, south Kalimantan, and south and north Sulawesi. A range of pests and diseases are found in these areas. Some pests such as white grub are specific to certain areas, while top and stalk borer are generally present in traditional sugarcane plantations. Some important diseases are already present in Indonesia such as ratoon stunting disease, smut, mosaic, pokkah boeng and leaf scald. Leaf scorch is a relatively new disease found in south Sumatra.

## Major Pests of Sugarcane in Indonesia

Improper treatment of cane cuttings before transporting them can cause the spread of not only diseases but insect pests to sugarcane areas formerly free from such problems. A report from Suhartawan (1992) indicated that eight important pests are present in Indonesian sugarcane plantations. They are top borer (*Tryporyza nivella*), stem borer (*Chilo saccharipagus*), giant borer

(*Phragmataecia castanae*), woolly aphids (*Ceratovacuna lanigera*), sugarcane scale (*Aulacaspis* sp.), white grub (*Lepidiota stigma*), rat (*Ratus argentiventer*; *Bandicota indica*) and feral pigs (*Babirousa babirussa*).

The top borer is present in the sugarcane plantations of Java, south Sumatra and south Sulawesi (Figure 1). The spreading of this insect was enhanced by the development of new sugarcane plantations outside Java in the 1980s following the shipment of large amounts of cane cuttings from east Java (Suhartawan 1992). Without any treatment, 90% of the stalk can be infested, causing about 9% sugar loss (Wirioatmodjo 1978).

Integrated pest management is believed to be the best way to control these pests. Some treatments such as cutting the top leaves showing the symptom of top borer infestation have been tried. However, they are not efficient because they are labour intensive and a large number of stalks die because of excessive cutting. Chemical treatments such as spraying or injecting the top with insecticide are ineffective for other reasons. Therefore, the use of a parasite is expected to solve the problem.

The situation with the stalk borer and the giant borer is different from that with the top borer. The stalk borer is already present in all sugarcane plantations in Indonesia (Suhartawan 1992), while the giant borer is present only in north Sumatra (Wirioatmodjo 1980). With the giant borer every percentage of stalk internode infestation can result in 0.75% sugar loss (Anon. 1979).

Woolly aphids and scale are present in all Indonesian sugarcane plantations. The population of the woolly aphids is influenced by the weather, especially the amount of rainfall (Samoedi 1993). During the dry season and heavy rainfall its population decreases. However, between September and December in Indonesia, the weather is conducive to its development. The impact of woolly aphid infestations on sugarcane plants depends on the growth stage of the

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plants. This aphid can reduce the sugar content by up to 30%.

Although scale is present in all Indonesian sugarcane plantations, its effect is relatively low. This may be because of parasites and predators. Clones with persistent and adherent leaf sheath are less tolerant.

White grubs are generally present in sugarcane areas with light soil consisting mostly of sand, as in some areas in Java (Kediri and Yogyakarta) and the south Kalimantan area of Pelaihari. Some different species of white grub were found in the Cintamanis and Gunung Madu Plantations of Lampung (south Sumatra).

Rats are an important pest in sugarcane plantations located close to rice producing areas — for example, near Cirebon (west Java). Rat infestation is usually heavy during a long dry season when there is little food from rice fields because water for rice cultivation is limited. Such pests are usually controlled chemically.

### Major Diseases Currently Present in Indonesia

Smut (*Ustilago scitaminea*) is currently the most important disease for sugarcane in Indonesia (Table 1), as reported from all Indonesian sugarcane plantations except in north Sulawesi (Paguyaman) and south Kalimantan (Pelaihari). Its presence in Indonesia was first reported in 1929 when the wonder cane (POJ 2878) was attacked by the disease. For a long time after that, no report of the disease was received (Handojo 1984). In 1979 it was reported that the disease was present in the north of central Java on the Trangkil sugar estate (Tjokrodirjo 1981). In this area, Suwarno et al. (1990) indicated that after ten years its presence was considered minor due to the use of resistant clones. However, Suryani et al. (1991) reported that smut had spread to

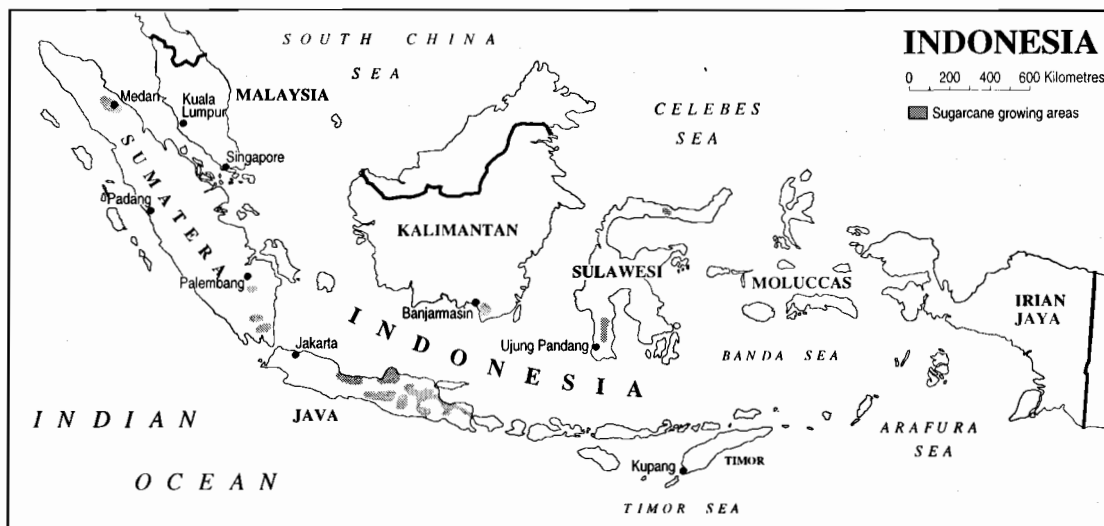
all sugarcane plantations in Indonesia and they presumed that its development was related to the length of the dry season in Indonesia.

**Table 1.** Major diseases in Indonesian sugarcane plantations for the past five years.

No.	Disease	Sugarcane plantation area
1.	Smut	All areas except south Kalimantan and north Sulawesi
2.	Ratoon stunting	All areas
3.	Leaf scald	North and south Sumatra including Lampung, Java
4.	Mosaic	All areas
5.	Leaf scorch	North-west and south Sumatra including Lampung
6.	Yellow spot	All areas
7.	Rust	All areas

The second most important disease in Indonesia is ratoon stunting disease. This disease is present in all Indonesian sugarcane plantations. It was first reported in Indonesia by Han (1960). A survey by Legowo et al. (1978a,b) indicated that the disease was found in most sugar estates throughout Java, with a relatively high infection rate. The disease can greatly reduce the sugarcane yield, especially when the dry period is quite long. Treating cane cuttings with hot water at 50°C for two hours is useful for controlling the disease. Handojo et al. (1978; 1981) indicated that such treatment of top cuttings can increase cane yield by more than 9% compared with the yield of untreated cuttings.

Leaf scald, which is caused by *Xanthomonas albilineans*, is another important disease present in Java, and north and south Sumatra. This disease is important



**Figure 1.** The Indonesian archipelago and the location of sugarcane plantations.



when the sugarcane is susceptible to such a disease and is dangerous when infected cane cuttings are brought to different areas (Han 1964). Using resistant clones and disinfecting the knives used for cutting the cane are believed to be the best ways of controlling the disease.

Mosaic disease caused by the sugarcane mosaic virus (SCMV) is present in all Indonesian sugarcane plantations. It is even in the area where sugarcane was planted as chewing cane (Suwarno 1989) on Madura, an island located close to Surabaya (east Java). Gillaspie et al. (1986) reported that there are seven types of SCMV in Java. With a certain strain of SCMV on the POJ3016 clone, the disease could reduce the sugar yield by 10–22% when the infection rate was more than 50% (Handojo et al. 1978; Darmodjo 1988).

Leaf scorch disease caused by *Stagonospora sacchari* is relatively new to Indonesian sugarcane plantations. Its presence was first reported by Handojo et al. (1986) in the Gunung Madu Plantations of Lampung, south Sumatra. This disease has caused large losses in Taiwan (Lo 1961). In Indonesia this disease quickly became important, especially in sugarcane areas around south Sumatra, because cuttings were frequently moved among sugarcane estates. Suranto (1988) and Suranto and Harsanto (1989) reported that sugar losses due to this disease on some susceptible clones such as Ragnar and SP70-1284 could range from 17% to 37%, depending on the age of the plants when they were infected. Suranto and Harsanto also reported different susceptibilities of some clones in different countries such as Taiwan, Indonesia and the Philippines. Such differences may be related to different environments and strains present in those countries.

Formerly, rust disease caused by *Puccinia kuehnii* was not considered important in Java (Han 1964), possibly because of the relatively dry conditions there compared with those of the islands of Sumatra and Kalimantan. However, with the development of new sugar estates, in Sumatra especially, a different type of rust disease caused by *P. melanocephala* was found (Handojo et al. 1986). This disease is known to be more dangerous than the one caused by *P. kuehnii*. On Q90, it resulted in sugar losses of 12–13% (Suranto et al. 1992).

New diseases or pests could occur in new sugarcane areas with the shipment or uncontrolled introduction of cane cuttings from outside those areas. In Indonesia, the development of new sugarcane plantations and estates outside Java needs a large amount of planting material that could be obtained easily from Java. However, uncontrolled shipment of the materials could also spread pests and diseases to the new areas. Therefore, some rules have been applied to prevent this. Technically, the rule is similar with that of exporting or importing planting materials from or into Indonesia. The difference is that cane materials transported

among Indonesian islands do not need to be quarantined, while imported planting materials need to be quarantined for a certain period.

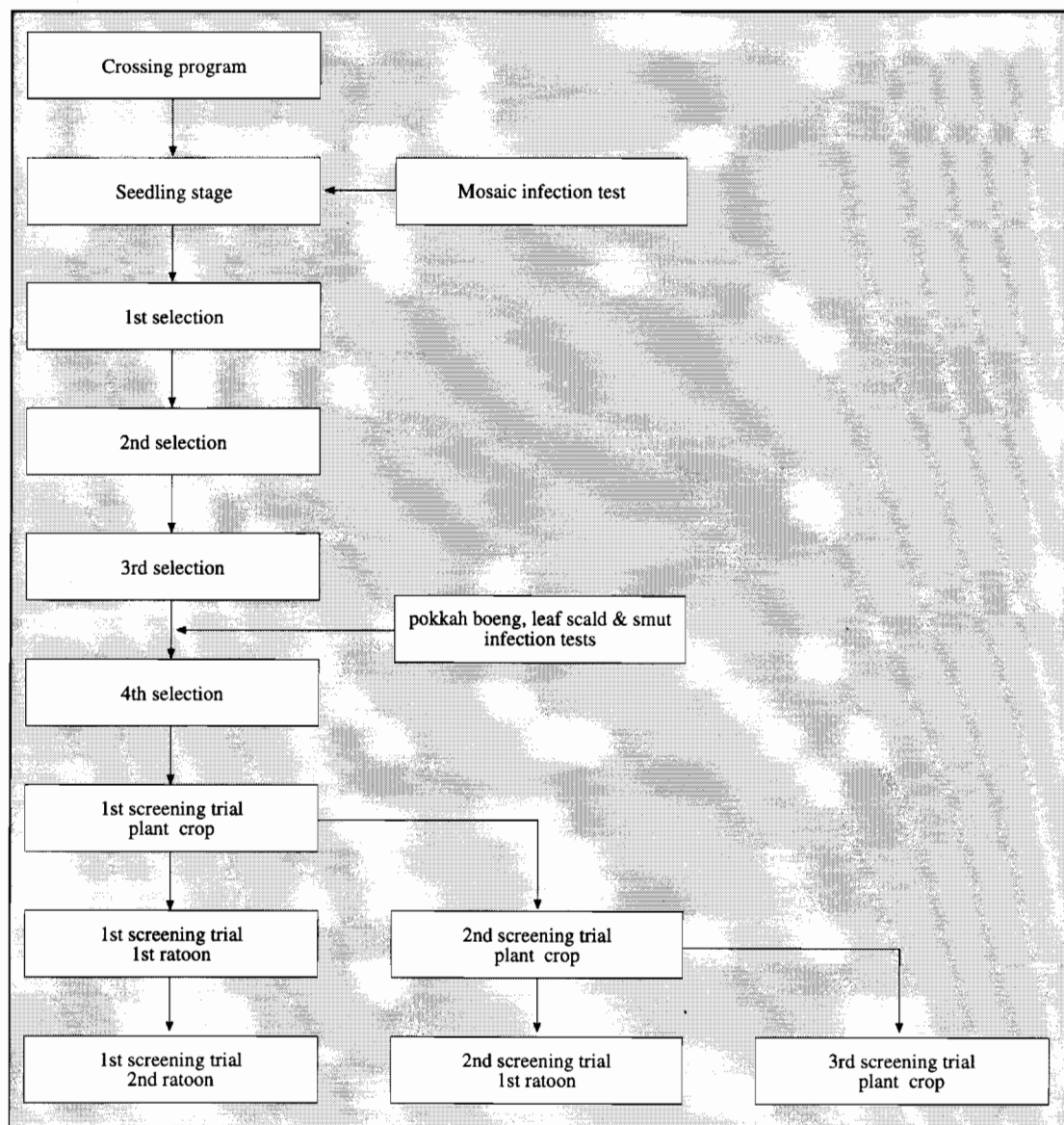
To prevent the introduction of new pests and diseases into sugarcane areas in other parts of Indonesia, all planting materials from overseas are brought to the quarantine station at Puteran Island near Madura in east Java. A certificate stating that the planting materials are clear of pests and diseases must accompany materials coming from overseas before entering the destination port in Indonesia. After being observed for any pest and disease for two years, clean materials can be sent to Pasuruan. A few diseases have been found in imported cane material while being observed in quarantine. They were mosaic, smut, leaf splitting and one with a symptom similar to gumming disease. All materials showing such symptoms were destroyed.

Exports of plant materials need to follow some procedures to prevent the spreading of Indonesian pests and diseases to other countries. The materials to be exported must be observed by the Indonesian Sugar Research Institute's entomologist and pathologist to make sure that there are no pests and diseases present. Healthy cuttings are treated with hot water at 52°C for 30 minutes, and then dipped in fungicide (for example, Benlate) before being packed for shipment. The pathologist issues a certificate of health to accompany those materials. Before such procedures can be done, a letter of permission for exporting must be obtained from the Minister of Agriculture.

### Developing Clones Resistant to Important Pests and Diseases

The development of the Indonesian Sugar Research Institute more than 100 years ago was enhanced by the presence of sugarcane diseases that were very dangerous to the sugarcane industry in Java. Therefore the Institute is quite familiar with the incorporation of resistance testing in the breeding program to develop clones resistant to certain diseases.

Before 1990 testing to obtain clones resistant to mosaic, pokkah boeng and leaf scald diseases was the only job done in the breeding program. The seedling population is infected with SCMV when the seedlings are about six weeks old and selection is carried out a few weeks later. Such a testing procedure is also conducted on the population of breeding materials selected from stage 3 of the selection scheme (Figure 2) before they go to the first yield trial. Therefore testing for resistance to mosaic disease is done twice during the selection process. Testing for clones' resistance to other diseases such as pokkah boeng and leaf scald is conducted on clones selected from stage 3 of the selection scheme. Therefore testing for resistance to such diseases is carried out only once in the breeding program and all of these activities are conducted



**Figure 2.** Schematic representation of the sugarcane selection scheme incorporating some disease infection tests to select resistant clones.

in Pasuruan. As a result, most of the clones released are resistant to the three diseases.

The development of new important diseases in Indonesia such as smut, ratoon stunt and leaf scorch resulted in some changes in the breeding program as well as the testing program in searching for clones resistant to such diseases. The development of clones resistant to ratoon stunting disease has not been accomplished since long hot water treatment is a relatively cheap way of controlling the disease compared

with the breeding program. However, since not all sugar estates have a facility for the hot water tank, it is difficult to stop the disease spreading.

Selection for resistance to smut disease is conducted in the Cirebon area of Jatitujuh in west Java, since the problems with the disease in that area were considered major. As reported by Lamadji et al. (1993) and Lamadji (1994b), preliminary evaluation indicated that 64 clones of *Saccharum* hybrids and one *S. officinarum* were resistant to smut. These resistant clones have

been used as parent materials in the Institute's crossing program. Seeds obtained from the crossing program were sown in Jatitujuh. Subsequently, selection was conducted in the area where smut disease was prevalent, while artificial infection by dipping cane cuttings in water containing spores of *Ustilago scitaminea* was conducted after stage three of the selection scheme.

Leaf scorch is found and prevalent only in south Sumatra. Therefore, selection to find clones resistant to the disease is conducted in that area. There are two centres for early selection — at the Bunga Mayang and the Gunung Madu sugar estates. Both are located in Lampung, south Sumatra. In this case seeds resulting from crossing are also sent from Pasuruan.

Sukarso and Wirioatmodjo (1984) proposed a breeding strategy for top borer resistant clones at the Indonesian Sugar Research Institute. Before that, Darmodjo (1975) indicated that the tolerance of clones to top borer was related to the top fibre content. Therefore, some foundation for breeding for resistance to top borer had been laid down. However, due to difficulties in rearing the insect, *Tryporiza nivella*, the program has not been implemented as part of the Institute's daily activities.

## Conclusions

Some important pests and diseases are already present in Indonesia. New pests and diseases in new sugarcane plantations outside Java could be introduced by improper handling and shipment of cane cuttings among islands and from overseas. Therefore, the rules for transporting cane cuttings among Indonesian islands and from overseas must be obeyed.

The Indonesian Sugar Research Institute has had a testing program to develop disease resistant clones since it was established. The sugarcane breeding program has been adjusted because of the presence of new important diseases in Indonesia by testing the newly developed seedlings in the infested areas. Some thoughts on breeding for borer resistance have been raised, but no strategy has been put into practise.

## Acknowledgment

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# Disease Constraints Associated with the Movement of Sugarcane Germplasm to and from Mauritius

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## Abstract

Sugarcane bacilliform virus is considered to be the main constraint in germplasm transfer from Mauritius. No curative measure is known and the virus has been detected in 125 noble canes (*Saccharum officinarum*), 17 interspecific hybrids as well as wild canes (*S. barberi*, *S. robustum*, *S. spontaneum* and *Ripidium* spp.). The disease is also a hindrance to the introduction of clones and, since 1989, 26 out of the 240 imported varieties were found to be infected. Ratoon stunting disease ranks second in importance among the diseases intercepted and has been detected in 10 clones in three quarantine cycles. An unidentified disease causing a reddish leaf mottle and death of older leaves was encountered in 1994 in six clones undergoing quarantine. Rod-shaped virus particles were found to be associated with the disease.

THE EXCHANGE of sugarcane plant material needs to be tightly regulated because of several systemic diseases. The general procedure for the quarantine of sugarcane has been reported by Gillaspie (1989) and guidelines for the safe movement of germplasm have been elaborated by Frison and Putter (1993). The necessity for extreme caution in transferring germplasm has been demonstrated in recent years with the discovery of several new diseases of viral origin — namely, Ramu stunt (Waller, Egan and Eastwood 1987), sugarcane bacilliform badnavirus (Lockhart and Autrey 1988), red leaf mottle (Baudin and Chatenet 1988), sugarcane mild mosaic clostero-like virus (Lockhart, Autrey and Comstock 1992), yellow leaf syndrome (Comstock, Irvine and Miller 1994) and a reovirus similar to Fiji disease reovirus (Bailey, these Proceedings, pp. 126–127). However, the development of sensitive techniques has contributed to more efficient disease diagnosis, so that the detection of diseases in quarantine is likely to increase in the coming years.

## Exports of Germplasm

In the past ten years, Mauritius has exported 336 varieties to 14 countries (Ramdoyal and Domaingue 1994). In the selection of cuttings for dispatch, attention has to be paid to gumming (*Xanthomonas campestris* pv. *vasculorum*), leaf scald (*Xanthomonas albilineans*), ratoon stunting (*Clavibacter xyli* ssp. *xyli*), smut (*Ustilago scitaminea*), sugarcane bacilliform badnavirus, sugarcane mild mosaic clostero-like virus and chlorotic streak (causal agent unknown) that are of quarantine importance.

Ratoon stunting, leaf scald, smut and chlorotic streak are eliminated by heat treatments and are not a cause for concern except if highly susceptible varieties are requested. Interspecific hybrids free of gumming disease can also be obtained owing to the limited distribution of the pathogen under present conditions. However, several noble canes (*Saccharum officinarum*) are highly infected by race 1 of the bacterium and it has not been possible to free these clones from the disease.

Sugarcane mild mosaic has been detected as a co-infection with the sugarcane bacilliform virus (SCBV) in 23 noble canes and 2 interspecific hybrids. A screening of clones in the germplasm collection tends to indicate that it is of limited distribution. SCBV is considered to be the major constraint on exports of germplasm from Mauritius. All of the 125 noble canes found in the collection are infected by the virus (Autrey et al. 1995). The virus has also been diagnosed in 17 interspecific hybrids, and out of 18 wild canes examined it has been detected in *Saccharum barberi*, *S. robustum*, *S. spontaneum* and *Ripidium* spp. Fluctuations in the level of the virus in the same stalk have been observed and it is possible that SCBV could go undetected at the time of dispatch. Varieties M 1236/71 and M 50/75 were reported to be infected by SCBV when these clones were exported.

## Imports of Germplasm

About 60–70 varieties are introduced to Mauritius every two years from various countries and during the past ten years 265 varieties have been imported from 16 countries and used mainly for breeding purposes. Owing to potentially damaging diseases such as Fiji and downy mildew, Mauritius takes advantage of the intermediate quarantine facility at Montpellier to

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transfer clones from countries where these diseases are present. There is also concern about sugarcane mosaic potyvirus (SCMV) as Mauritius is one of the two countries where the disease does not exist (Koike and Gillaspie 1989).

### Quarantine system

A quarantine system for sugarcane in close confinement was established in 1928 (Wiehe 1960). The introduction of related grasses is strictly controlled and only cuttings of sugarcane have been imported, not true seed. Clones are introduced according to phytosanitary conditions laid down by the Ministry of Agriculture and Natural Resources. The maintenance and quarantine of the plants are carried out by the Mauritius Sugar Industry Research Institute and the operational procedures have been described by Autrey, Dhayan and Sullivan (1990). The facility is situated at Réduit and one quarantine cycle lasts 18–20 months. It consists of one plant cane and one ratoon crop of equal duration prior to one year open quarantine on the Institute's premises.

### Disease detection

Disease detection is based on symptomatology and techniques such as light and immunofluorescent microscopy, electron microscopy and enzyme-linked immunosorbent assay. Often diagnosis is requested from foreign institutions such as IACR, Rothamsted, UK, Natural Resources Institute, UK, or the University of Minnesota, USA.

In plant cane, six months after establishment, all clones are examined by electron microscopy. Each stalk is visually inspected at harvest and suspected cases investigated by an appropriate technique. Any infected clone is immediately destroyed. The same

procedure is repeated in first ratoon and, if found disease-free, 10–15 two-eye cuttings of each variety are planted in open quarantine after being subjected to the dual hot water treatment or the cold soak, long hot water treatment.

### Interception of diseases in quarantine

Diseases other than SCBV or unidentified problems encountered in quarantine are summarised in Table 1. Disease interception in quarantine was relatively rare when the system was first established. In the late 1950s spindle inoculations in variety BH 10/12 was carried out in quarantine to detect SCMV (Wiehe 1960). There is no report on the detection of the virus by this method but in 1965 variety 51 NG 88 was destroyed because it displayed symptoms suggestive of mosaic (Mauritius Sugar Industry Research Institute 1966). In 1994, particles of a potyvirus were detected in variety CP 71 1038. No symptom was present and SCMV could not be confirmed upon further investigation.

Ratoon stunting has been detected in ten varieties in three quarantine cycles and is the most important disease, after SCBV, to be intercepted.

Since 1989, all varieties have been systematically inspected by immunosorbent electron microscopy for SCBV. Of the 240 varieties tested, 26 were found to be infected and were destroyed (Table 2).

In November 1994 a pronounced red mottling was observed in the first ratoon of variety CP 85 845 (HoCP 85 845). The symptoms were attributed to abiotic factors such as deficiency or temperature effect that are observed on sugarcane grown under glass-house conditions. One month later, the symptoms increased in intensity and the old leaves turned necrotic and died (Figure 1). Four other varieties — CP 70 324, CP 71 1240, CP 88 1508 and CP 88 1540

**Table 1.** Diseases and unidentified problems intercepted on sugarcane clones undergoing quarantine.

Cycle	Country of origin	Varieties	Nature of problem
1960–62	Barbados	B 47258	Unidentified leaf striping
1962–64	India	Co 475	Clustered stool symptoms
	USA	CL 41-142	Rotting of base
1964–66	Hawaii	51 NG 88	Suspected mosaic
1975–77	USA	CP 68-1026	
	Taiwan	F171	Ratoon stunting
	Australia	Hébé	
	Brazil	CB 45-3	Basal stem rot
1979–81	Indonesia	IJ 76-428	Frecklings
1985–87	Taiwan	ROC 1, ROC 2, ROC 3, ROC 5	Ratoon stunting
	Philippines	VMC 73-229	
1987–89	Barbados	BT 7230, BT 57441	Ratoon stunting
1993–95	USA	CP 70 324, CP 71 1240, CP 85 845, CP 87 1274, CP 88 1508, CP 88 1540	Red mottling, drying of leaves and association of rod-shaped virus particles
	USA	CP 71 1038	Potyvirus



**Table 2.** Clones infected by sugarcane bacilliform virus intercepted in quarantine

Countries	Quarantine cycles								Total	
	1987-89		1989-91		1991-93		1993-95		1987-95	
	+	-	+	-	+	-	+	-	+	-
Argentina	1	3	NC	NC	NC	NC	NC	NC	1	3
Australia	0	10	NC	NC	1	3	NC	NC	1	13
Barbados	1	4	0	8	NC	NC	NC	NC	1	12
Brazil	4	9	0	12	0	10	0	6	4	37
Colombia	NC	NC	0	11	NC	NC	NC	NC	0	11
India	NC	NC	NC	NC	NC	NC	0	2	0	2
Mexico	NC	NC	0	5	NC	NC	0	6	0	11
Puerto Rico	1	2	0	3	2	1	0	2	3	8
Réunion	1	7	2	9	4	4	4	8	11	28
South Africa	0	1	0	6	0	4	0	5	0	16
Taiwan	0	3	0	2	0	1	0	1	0	7
USA	1	15	0	14	2	9	2	28	5	66
Total	9	54	2	70	9	32	6	58	26	214

+ Number of infected clones. - Number of clones not infected. NC No clones imported from that country.



**Figure 1.** Red mottling, leaf curling (1) and necrosis of lower leaves (2) in variety HoCP 85 845 in the cane quarantine glasshouse. Rod-shaped virus particles (3 & 4) have been found to be associated with the symptoms (micrograph from Mr R. Woods, IACR, Rothamsted).

— showed the same symptoms and an examination of leaf extracts by electron microscopy revealed stiff tobacco mosaic virus-like particles that were confirmed by Mr R Woods, IACR, Rothamsted (Figure 1). The other varieties were kept under observation and six months later variety CP 87 1274 developed the same symptoms. The disease could possibly be related to red leaf mottle caused by the peanut clump furovirus and is being investigated at IACR, Rothamsted. Field surveys carried out to determine the occurrence of such a disease in Mauritius have been negative.

## Conclusions

As it is not possible to predict the significance of a plant disease if introduced to a country, its transfer must be intercepted. The holding of imported material in isolation until it is believed to be healthy is therefore essential, even though this period may be quite long, as it is for sugarcane. However, given the high humidity, high temperature, temperature fluctuations and lack of sunshine that are normally encountered in glasshouses, a disease may not express itself as it would normally have done in the field. Variations in symptoms may also occur. It is therefore important to know the conditions that would optimise disease expression in the glasshouse and there is a need to obtain such information for sugarcane.

There are two approaches adopted by countries for the quarantine of sugarcane: observations of a plant crop and one ratoon or of two plant crops with hot water treatment in between. Both have their merits and Mauritius has adopted a 'detect and destroy' strategy as opposed to a 'cure and release' one, as the hot water treatment is not foolproof and most viruses can survive the treatment. The development of molecular biology techniques that would enable detection of a low level of infection in the absence of symptoms is expected to be a tremendous leap forward. At present, few sugarcane diseases can be detected by this means — namely, SCMV and Fiji (Smith and Van de Velde 1994), SCBV (Braithwaite et al. 1994) and ratoon stunting (Chung, Lin and Chen 1994). Once such techniques are established, the exchange of such tools should be beneficial for the safe movement of sugarcane.

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# Major Diseases Affecting Sugarcane Production in India and Recent Experiences in Quarantine

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## Abstract

Several major and minor diseases caused by fungi, bacteria, viruses, mycoplasma and nematodes affect the growth of sugarcane in India. The diseases of economic importance in India are red rot, smut, wilt, grassy shoot, ratoon stunting and leaf scald. Red rot disease is still considered the major threat to sugarcane cultivation on the east coast and in subtropical parts of India. Variability within the red rot pathogen is a major constraint on the evaluation and identification of resistant clones and management. Smut disease is causing severe losses to standing canes in Karnataka and Maharashtra States. Wilt disease is attaining significance in many parts of India, particularly in South Gujarat. Grassy shoot, ratoon stunting and leaf scald diseases, although considered less important diseases, attained damaging levels in many parts of the country. Foliar diseases also cause losses in some locations. In India, quarantine between states is not being followed strictly.

SUGARCANE is cultivated in India in the tropical belt comprising the States of Maharashtra, Gujarat, Karnataka, Andhra Pradesh, Kerala and Tamil Nadu and the subtropical belt comprising the States of Punjab, Haryana, Uttar Pradesh, Bihar and Orissa. The planting season is from February to March in the subtropics and from January to February in the tropics. The soil in the subtropics is mostly alluvial and in the tropics clayey, loamy or sandy loam. In India sugarcane is cultivated on more than 3 million hectares for the manufacture of white sugar, Gur and Khandsari.

Vegetative propagation encourages the accumulation and carryover of some pathogens in seed pieces. The factors that favour the prevalence of diseases are the continuous cultivation of sugarcane over large areas, the presence of the crop throughout the year, the genetic uniformity of the cane, the climate of tropical and subtropical regions and the unrestricted movement of seed material from one region to another. A conservative estimate of the total loss due to diseases of sugarcane ranged from 10% to 15% (Alexander 1982). However, total crop loss was seen in coastal Tamil Nadu due to a severe red rot outbreak recently.

Though breeding strategies are being oriented toward development of disease resistant genotypes they have had limited success. Similarly several disease management measures have had limited success under field conditions.

## Red Rot

Red rot is caused by a fungus *Colletotrichum falcatum* Went (Perfect stage *Glomerella tucumanensis* Arx and Muller). In India, red rot is mainly a disease of the standing stalk. It is the most dreaded disease of sugarcane and for several years has been posing a serious threat to sugarcane growers and the sugar industry. The disease is widely prevalent in subtropical and eastern coastal regions in India. Recently severe red rot outbreaks have been recorded in sugarcane tracts of South Gujarat and interior parts of Tamil Nadu. Recent epidemics in Tamil Nadu, Andhra Pradesh, Orissa and Gujarat resulted in the loss of high sugar varieties such as CoC671, CoC8001, CoC85061, CoC86062, CoC92061 and other varieties such as Co658, Co419, Co785, Co997 and Co6304. Large losses resulted from the death of cane stalks, the low extraction of juice and a reduction in sucrose content. Diseased stalks used for planting show extremely poor germination.

Physiological races of the red rot pathogen are known to occur (Abbott 1936). Recently the Institute's work showed the existence of many pathotypes in different agroclimatic zones (Alexander et al. 1993). A comparison of different pathotypes showed that possibly five races of *C. falcatum* existed. The races were from the varieties Co1148, Co7717, Co419, Co997 and CoC671. Until the early 1970s breeding for red rot resistance was carried out based on screening genotypes to the most virulent isolates of the pathogen. However, this type of rating was not uniform and reaction varied at each endemic location, depending on the composition of local red rot flora (Alexander and Rao 1972).

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Alexander et al. (1976) initiated breeding for polygenic or horizontal resistance, and this produced 33 varieties with acceptable commercial attributes and with resistance to two or three pathotypes.

### Smut

Smut disease is incited by *Ustilago scitaminea* Syd. Smut disease is endemic in most parts of the sugarcane growing tracts and more prevalent in the tropical areas. The disease is severe in neglected ratoons. Infections build up to higher levels in ratoons if the disease in the plant crop is neglected. Severe epidemics of the disease have occurred recently, very frequently in ratoons of Co740 and Co7527 in Maharashtra State. Cane yield and quality loss have been reported due to smut disease (Alexander 1986).

The disease favours a warm and dry environment. Moderate rainfall and maximum temperatures of 34°C favour a high incidence of the disease (Saxena and Singh 1966). As there is evidence of only one major race of smut in India, biparental crossing involving a resistant parent is suitable for obtaining the desired resistance in the progeny. Smut resistant mutants of Co740 and Co419 and smut resistant somaclones of CoC671 have also been obtained. Smut disease increases slowly in the field and it can be managed easily by following phytosanitary practices, ploughing out ratoons in severe cases and heat treatment in combination with a systemic fungicide (Padmanaban 1984).

### Wilt

Wilt disease has been reported to be a complex disease involving *Cephalosporium sacchari* Butler and *Fusarium moniliforme*. *Acremonium* species that may be synonymous with *Cephalosporium* is also reported. Two species of *Acremonium*, namely *A. furcatum* and *A. terricola*, are reported to be associated with wilt (Singh and Singh 1974). In recent studies the nematodes *Helicotylenchus*, *Pratylenchus*, *Hoplotaimus* and *Tylenchorhynchus* sp. were reported to be associated with the disease along with fungi. The nematodes were found to increase the incidence of the disease. The disease expression is conditioned by favourable weather and soil factors such as pre-monsoon drought followed by excessive moisture in the post-monsoon period.

Wilt was reported to be endemic in Bihar and is now present in most of the states. Many varieties such as Co527, Co951, Co1007, Co245 and CoS321 went out of cultivation due to wilt. The disease affected Co419, Co527, Co975, Co997 and CoC671 in Andhra Pradesh in different epiphytotic. In South Gujarat the disease appeared in an epiphytotic proportion in 1985, mainly affecting Co6304 and CoC671 in Bardoli, Chalthan, Madhi and Gandevis factory areas. In many fields 60–71% of the cane was affected. In this case root borer was associated with wilted canes.

Under field conditions wilt is often associated with red rot, pineapple disease or scale insects. Synergism of red rot and wilt is not unknown and at times becomes far more destructive than either of the two. High soil moisture and rainfall increase the disease severity. Moderately high temperature (26–28°C) promotes infection and disease development. Moisture stress and high day temperatures during summer months adversely affect resistance to the wilt pathogens (Sharma 1976). Currently wilt disease is increasing at an alarming rate. The mechanisms of wilt resistance are not well understood and studies along these lines are needed.

### Pineapple Disease

Pineapple disease caused by *Ceratocystis paradoxa* Moreau is a disease of stem pieces during the germination phase of the crop. The disease is prevalent in most of the sugarcane tracts, particularly in the tropical belt. The disease is accentuated in poorly drained fields and heavy soils. The soil-borne pathogen affects the germination of seed cane, which leads to lower numbers of millable canes per unit area and poor yield. The disease can be effectively controlled by 0.1% carben-dazim dip of setts before planting.

### Ratoon Stunting Disease

Ratoon stunting disease was first reported in 1956 in India and built up to alarming proportions in varieties such as Co419, which was ultimately withdrawn from cultivation in Karnataka. That variety, which is normally a thick cane, was observed to be only pencil thick in Gangavati areas in Karnataka in the 1980s. The disease slowly builds up in canes and the cane ultimately becomes stunted with no apparent external symptoms. It is present throughout the sugarcane growing areas of this country. Heat treatment and nursery programs effectively control the disease.

### Leaf Scald Disease

Leaf scald is a bacterial disease, which was first observed in India in 1962. This disease is a serious problem in varieties such as BO70, Co419, Co1148, Co7304 and CoS659. Recently this disease was seen in the variety CoC90063 in different intensities in Nellikuppam areas of Tamil Nadu. Leaf scald is caused by *Xanthomonas albilineans* (Ashby) Dowson. It displays various symptoms depending on the growth conditions of the cane. The disease may be present in a variety in chronic or acute forms. In severe outbreaks yield losses may exceed 25%. The yield and juice quality are substantially reduced by the extensive sprouting of nodal buds. The environmental conditions that favour the disease's development are drought, low temperatures, soil with a poor nutrient status and stagnant water.

## Grassy Shoot Disease

Grassy shoot disease is caused by mycoplasma-like organisms. In India the disease was first observed in 1919 (Vasudeva 1955). It has spread to all the sugar growing areas of this country. In many areas it is next in importance to red rot and smut. Recently the disease has gained importance in Tamil Nadu, Karnataka and Andhra Pradesh, 10–15% incidence in fields being common. Many valuable varieties such as Co419, Co453, Co740, Co975, Co1148, CoJ46, CoS510, CoC671, Co8021 and CoC92061 suffer from this disease. Though primary spread of the disease is through infected setts the mechanism of secondary spread of mycoplasma-like organisms is not known. The role of aphids and plant hoppers in transmitting the grassy shoot pathogen is still disputed (Samaddar, Singh and Varma 1977). No critical studies have been made of the effect of temperature, humidity and soil conditions on the disease's development.

## Other Diseases

Foliar diseases such as rust, yellow spot, eye spot, brown spot, brown stripe and ring spot caused by fungal pathogens often inflict severe damage on crops in some locations, depending on environmental and other factors.

## Quarantine

Many of the important diseases of sugarcane in India are sett borne. Vegetative propagation encourages the accumulation and transmission of the pathogens through seed pieces. In India seed pieces of various varieties of sugarcane are routinely exchanged between and within states for breeding purposes and commercial culture; no regulatory measures are imposed during such movements of seed cane. Many important pathogens and pests go unnoticed along with

the seed cane and get disseminated to new areas. In India the introduction of red rot and wilt to new areas with seed cane are examples of the importance of this type of disease spread. Recently changes have been made to correct this situation.

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# Major Diseases Affecting Sugarcane Production in Guadeloupe and Réunion Island, and Recent Experiences with Sugarcane Diseases in Quarantine at CIRAD-CA in Montpellier

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## Abstract

No major diseases are currently affecting sugarcane production to a large extent in Guadeloupe and Réunion Island at this time. However, numerous diseases, including rust, smut, leaf scald and ratoon stunting disease, are present, and constant observation and care in breeding programs are required. Leaf scald caused by *Xanthomonas albilineans* and mosaic caused by the sugarcane mosaic virus are the most frequent diseases encountered at CIRAD's sugarcane quarantine in Montpellier, France.

THE SUGARCANE PATHOLOGY PROGRAM of CIRAD is found in three different locations: Guadeloupe (West Indies) and Réunion Island (Mascareignes), two sugarcane producing areas, and Montpellier (France), a non-producing region where sugarcane is grown in quarantine greenhouses.

## Diseases of Sugarcane in Guadeloupe

No major diseases are presently affecting sugarcane production to a large extent in Guadeloupe. However, leaf scald caused by *Xanthomonas albilineans* was a major problem in the 1980s (Rott and Feldmann 1991). The cultivation in commercial fields of two cultivars (B69566 and B69379) susceptible to leaf scald was largely responsible for this occurrence. It was shown that leaf scald can affect yields in both cultivars but the progress of the disease differed in each. Cultivar B69566 could recover from the disease to a certain extent from one ratoon to another whereas cultivar B69379 could not (Rott et al. 1995). Leaf scald was brought under control by replacing B69379 with more resistant cultivars and by replanting fields with clean nursery-grown planting material.

Another disease was occasionally observed in the 1980s in Guadeloupe. It was characterised by a progressive wilting of the foliage and the death of isolated stalks. No pathogen was isolated from the affected stalks and the disease did not seem to cause any great

losses. The symptoms observed in Guadeloupe closely resembled those of dry top rot caused by *Ligniera vasculorum*, a disease recently described in Florida (Comstock, Miller and Farr 1994). The involvement of this pathogen in diseased sugarcane in Guadeloupe is under investigation.

Other major diseases in Guadeloupe include smut (*Ustilago scitaminea*), rust (*Puccinia melanocephala*) and ratoon stunting disease (*Clavibacter xyli* ssp. *xyli*). New cultivars developed by CIRAD's breeding program are screened in specific trials for resistance to smut and leaf scald. Cultivars susceptible to foliar diseases (for example, rust, red stripe, chlorotic streak, yellow spot) are eliminated during the selection process. Other diseases including ratoon stunting disease are controlled by the use of clean planting material produced in a nursery system where the mother nurseries are initiated with tissue-cultured plants (Feldmann and Rott 1991). Foreign cultivars are introduced in Guadeloupe following quarantine at CIRAD's facilities in Montpellier to avoid the introduction of new pathogens or new strains of pathogens that are already present.

## Diseases of Sugarcane on Réunion Island

The 1925 epidemic of mosaic virus was responsible for the creation of the breeding station CERF (Centre d'Essai de Recherche et de Formation). The disease was brought under control by the use of resistant cultivars, and further incidence has been negligible since the late 1930s.

Great outbreaks of gumming disease caused by *Xanthomonas campestris* pv. *vasculorum* occurred in 1925 and 1954. Only sporadic incidence has been reported since then. For example, an outbreak occurred in the early 1980s when cultivar S17, until

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then considered resistant to gumming disease, exhibited systemic symptoms in the uplands of the island. An outbreak of leaf scald disease was recorded in the early 1980s due to the cultivation of cultivar H328560. Yellow spot (*Mycovellosiella koepkei*) and chlorotic streak are frequently observed in highly humid zones but their effects on yield are unknown. Smut and rust are present but controlled by the use of resistant cultivars. Ratoon stunting disease has been diagnosed on Réunion Island but its incidence seems to be low.

More than 30 diseases have been recorded on Réunion Island but none are presently affecting sugarcane production to any major extent. A strict selection policy advocating disease resistance has been applied since the creation of CERF. Major diseases are controlled by the use of resistant cultivars. The new cultivars developed by CERF are screened for resistance to diseases by CIRAD. Sugarcane is inoculated in specific trials with the pathogens causing rust, gumming disease, leaf scald and smut. Foreign cultivars are introduced to Réunion Island following quarantine at local facilities at CERF or at CIRAD's quarantine greenhouses in Montpellier.

### Experiences with Sugarcane Diseases in Quarantine at CIRAD-CA in Montpellier

The French sugarcane quarantine facilities were created in 1965 to supply African countries with clean planting material. The quarantine was first located in Nogent sur Marne, close to Paris, but was moved to Montpellier in 1978 (Baudin 1984).

Sugarcane cultivars have various origins, including:

- freely accessible commercial cultivars or wild canes used in breeding programs from all over the world (Argentina, Brazil, Indonesia, Mexico, Réunion Island, USA, ...);
- cultivars developed by the West Indies Central Sugar Cane Breeding Station in Barbados; and
- cultivars developed by CIRAD-CA in Guadeloupe (FR clones) and CERF on Réunion Island (R and RP clones).

Sugarcane is observed and tested for diseases in Montpellier for two consecutive crop cycles of 9–12 months each. A long hot water treatment (soaking of cuttings for two days at ambient temperature followed by three hours at 50°C) is used between the two crop cycles, conforming to the recommendations of the International Board for Plant Genetic Resources and the International Society of Sugar Cane Technologists (Frison and Putter 1993). Expertise in diagnostics is also available at the same location.

Leaf scald and mosaic are the two most frequently encountered diseases in the first quarantine crop cycle at Montpellier. Symptoms of brown spot caused by *Cercospora longipes* were observed once in 1986, and a few cases of smut have also occurred. The infected plants were removed from the quarantine and destroyed as soon as the diseases were detected.

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# Major Sugarcane Diseases in Fiji

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## Abstract

Thirty diseases of sugarcane have been recorded in Fiji. However, only two are currently of economic significance. These are Fiji disease caused by a reovirus, and downy mildew caused by *Peronosclerospora sacchari*. Both are presently found on the island of Viti Levu. The principal method of controlling these two diseases is growing resistant varieties combined with intensive roguing. Ratoon stunting disease (*Clavibacter xyli* ssp. *xyli*) may also be a yield limiting factor but further research is required. The Fiji sugar industry is fortunate that smut (*Ustilago scitaminea*) and mosaic (a potyvirus) are not present. Thus the risks of introducing new diseases, due to increasing germplasm importation now and in the future, poses real hazards for sugarcane production in Fiji. Recommendations are made to lessen these quarantine risks at both national and international levels.

Fiji has a total land area of 1 822 921 hectares, comprised of 332 small islands. The two largest islands are Viti Levu (1 042 900 ha) and Vanua Levu (555 600 ha). Sugarcane is grown in the dry zones of these two islands, covering approximately 75 000 hectares of land with an average yield of 50–58 tonnes of cane per hectare. In 1994 the four mills of the Fiji Sugar Corporation crushed 4.06 million tonnes of cane, producing a record sugar yield of 516 589 tonnes. The present production system is based on four hectare smallholder farms.

The commercial growing of sugarcane began in the 1870s and sugar was Fiji's leading export by 1883 (Chandra 1983). Since then, no other product has displaced it. In the first six decades, gumming disease (*Xanthomonas campestris* pv. *vasculorum*, Fiji disease (Fiji disease virus, a reovirus) and downy mildew (*Peronosclerospora sacchari*) were the major diseases affecting sugarcane production. Gumming disease disappeared in the 1930s as Badila and POJ 2878 replaced the susceptible Malabar variety.

In 1934, mosaic disease (sugarcane mosaic virus, a potyvirus) was found in 19 stools of the Malabar variety in Nausori (Colonial Sugar Refining Co. 1934). No further occurrence of mosaic disease was recorded until 1988 when it was found in the Yaqara germplasm collection (Fiji Sugar Corporation 1988). Fortunately, mosaic did not become established. In addition, culmicolous smut (*Ustilago scitaminea*) is not present in Fiji. The record by Parham (1953) that smut was found on sugarcane in Fiji during the 1940–44 period is questionable. Ferreira and Comstock (1989) stated that by the end of 1983 only the sugar industries of Australia and Fiji remained free of smut. Excluding smut, thirty diseases have been recorded in sugarcane in Fiji (see Table 1).

Thus the Fiji sugar industry has only a few diseases of economic significance. The two most important diseases are Fiji disease and downy mildew, which at present are found only on the island of Viti Levu. Ratoon stunting disease (*Clavibacter xyli* ssp. *xyli*) could also be an important disease but further research is needed. Therefore this paper briefly discusses Fiji disease, downy mildew and ratoon stunting disease and their controls, combined with sugarcane germplasm and quarantine.

## Fiji Disease

Fiji disease is caused by a reovirus and transmitted by the leaf hopper *Perkinsiella vitiensis* Kirk. The main diagnostic symptom is the presence of galls on the undersurface of the leaf blade and stunted plant growth. The disease was first recorded in Fiji on commercial cane growing in the Rewa River district in 1886 (Robinson and Martin 1956). A devastating epidemic occurred that destroyed thousands of acres of sugarcane during 1906 (Daniels, Husain and Hutchinson 1971). A clean seed program and release of resistant varieties (Badila and Pompey) brought the outbreak under control.

The introduction of two susceptible varieties, Ajax and Pindar in the 1940s and 1950s, created another destructive epidemic. By the early 1960s at least 10% of the stools in a field had Fiji disease (Egan, Ryan and Francki 1989). Replacement of susceptible varieties with resistant ones (Ragnar, Homer and Spartan) combined with the implementation of an intensive roguing program, reduced infection to a low level.

Fiji disease at present is largely confined to cane in the Lautoka Mill area. In 1993 the disease was found in five cane sectors. The percentage of farms with Fiji disease ranged from 0.6% to 5.9%, with an average 2.7% per sector. The total number of infected stools rogued out in these sectors was 884. In 1994 there were

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**Table 1:** Diseases recorded in sugarcane in Fiji.

Year first recorded	Disease	Pathogen
1886	Fiji disease	Fiji disease virus, a reovirus
1895	Gumming disease	<i>Xanthomonas campestris</i> pv. <i>vasculorum</i> (Cobb 1893) Dye 1978
1910	Downy mildew	<i>Peronosclerospora sacchari</i> (T. Miyake) Shirai & K. Hara
1910	Leaf splitting disease	<i>Peronosclerospora northii</i> (Weston) C.Q. Shaw
1912	Pineapple disease	<i>Ceratocystis paradoxa</i> (Dade) Moreau
1915	Orange rust	<i>Puccinia kuehnii</i> Butl.
1915	Red rot	<i>Glomerella tucumanensis</i> (Speg.) Arx & Müller (con. stat. <i>Colletotrichum falcatum</i> Went.)
1921	Leaf scald	<i>Xanthomonas albilineans</i> (Ashby 1929), Dowson 1943
1929	Banded sclerotial disease	<i>Thanatephorus sasakii</i> (Shirai) Tu & Kimborough
1930	Eye spot	<i>Bipolaris sacchari</i> (Butl.) Shoemaker
1934	Mosaic	Sugarcane mosaic virus (SCMV), a potyvirus
1938	Rind disease	<i>Phaeocystotroma sacchari</i> (Ell & Ev.) B. Sutton
1938	Stem rot	<i>Athelia rolfsii</i> (Curzi) Tu & Kimborough (sclerotial stat. <i>Sclerotium rolfsii</i> Sacc.)
1938	Sheath rot	<i>Gibberella fujikuroi</i> (Sawada) Ito (con. stat. <i>Fusarium moniliforme</i> Sheldon)
1938	Root rot	<i>Marasmius sacchari</i> Wakker
1950	Pokkah boeng	<i>Gibberella fujikuroi</i> (Sawada) Wollenw. (con. stat. <i>Fusarium moniliforme</i> Sheldon)
1951	Yellow spot	<i>Mycovellosiella koepkei</i> (Krüger) Deighton
1953	Ratoon stunting disease	<i>Clavibacter xyli</i> ssp. <i>xyli</i> Davis et al.
1953	Chlorotic streak	Causal agent unknown
1954	Leaf splitting disease	<i>Mycosphaerella striatiformans</i> (Cobb) Sacc. & Trott.
1954	Brown stripe	<i>Cochliobolus stenospilus</i> (Drechs.) Mat. & Yam.
1959	Leaf scorch	<i>Stagonospora sacchari</i> Lo and Ling
1961	Sheath rot	<i>Cytospora sacchari</i> E. Butler
1961	Mottled stripe	<i>Pseudomonas rubrisubalbicans</i> (Christopher & Edgerton) Krasil'nikov
1963	Root rot	<i>Pythium arrhenomanes</i> Drechsler
1967	Red stripe	<i>Pseudomonas rubrilineans</i> (Lee et al.) Stapp
1970	Ring spot	A fungus, probably <i>Leptosphaeria sacchari</i> B. de Haan
1978	Brown spot	<i>Cercospora longipes</i> Butler
1983	Dry rot	<i>Physalospora rhodina</i> (Berk. & Curt.) Cke
1984	Common rust	<i>Puccinia melanocephala</i> H. & P. Sydow

eight sectors with the disease and the average percentage of farms affected per sector was 3.5%. A total of 1158 infected stools were rogued. A new outbreak of Fiji disease occurred in the Veisaru sector of the Rarawai Mill area in 1994. It has been observed that most Fiji disease infestations occur in areas where *Saccharum edule* Hassk. (duruka) is planted. Duruka, an alternative host, is consumed by native Fijians as a vegetable. To eradicate duruka in these areas could create a social problem in the industry.

### Downy Mildew

Downy mildew, caused by the fungus *Peronosclerospora sacchari* (T. Miyake) Shirai & K. Hara, was first detected in Fiji at the Rarawai Mill in 1910

(Robinson and Martin 1956). The main diagnostic symptom is pale to light yellow leaf streaks parallel to the midrib separated by normal green tissue. It was believed that the fungal pathogen was introduced to Fiji from Papua New Guinea via Australia (Daniels et al. 1971). Within two years the disease had spread to all mill areas except Nausori. Rose Bamboo and Striped Yellow Singapore were the two main cane varieties grown at that time (Daniels et al. 1971). The disease was controlled by the use of resistant varieties (Badila and Pompey) and by roguing from 1913 to 1930. However, due to the introduction of susceptible varieties such as P.O.J. 2878, Argus and Eros, devastating epidemics occurred during the 1930s and 1940s. Again these susceptible varieties were replaced with resistant ones such as Mentor, Pindar and Ragnar in the

early 1950s. However, with the 1960s release of the variety Waya, which is moderately susceptible to downy mildew, losses from the disease have continued.

The growing of resistant varieties combined with the use of clean seed and an intensive roguing system have reduced downy mildew infection to a low level. At present, the disease is found only in the Olosara sector. Twenty per cent of the farms in this sector have the disease and the total number of infected stools rogued last year was 3469. The percentage of stools infected was highest on farms planting an unapproved variety called Raki-Kaba. Maize, an alternative host, is a major cash crop in this sector and its close proximity to sugarcane has aided the rapid spread of the disease during the cane growing season.

### **Ratoon Stunting Disease**

Ratoon stunting disease is caused by the bacterium *Clavibacter xyli* ssp. *xyli*. Stunting, unthrifty growth and internal stalk (nodes) discolouration are the main diagnostic symptoms. Slides containing cane saps, sent to R.A. Bailey (SASEX) in 1994, confirmed the presence of ratoon stunting disease in Fiji. However, the disease was first identified in Fiji in 1953 at Nausori, Rarawai and Lautoka (Colonial Sugar Refining Co. 1953). Its present distribution and impact on cane yield are not known. Ratoon stunting disease may be an important yield limiting factor in some areas of the Fiji sugar industry. As a precautionary measure, a hot water treatment plant has been installed at the Sugarcane Research Centre (Lautoka) for treatment of seed cane especially against ratoon stunting disease and other seed-borne diseases.

### **Screening Sugarcane Varieties for Disease Resistance**

Planting of resistant varieties remains the principal method of controlling Fiji disease and downy mildew in Fiji. The breeding and screening of new varieties for disease resistance are therefore ongoing processes. Most of the fourteen current recommended varieties are resistant to Fiji disease and downy mildew.

An insectary method for screening sugarcane varieties for resistance to Fiji disease was developed in the 1960s to overcome the problems faced with field trials (Husain and Hutchinson 1971). It involves exposing test plants to viruliferous leaf hoppers in cages and recording the latent period. This nursery technique assumes that the susceptibility of a variety is inversely correlated to the latent period — that is, the longer the latent period the more resistant the variety. In Fiji this method has been found to be faster, less expensive and more reliable than field trials.

Similarly, screening sugarcane varieties for resistance to downy mildew is conducted in a disease nursery within the Tavakubu pine forest. Test varieties in

pots at 2–3 leaf stage are placed between rows of heavily infected susceptible plants (Eros and local maize) for natural inoculation. After 14 days, the test plants are removed from the inoculation site and placed in another area for observation of disease symptoms.

### **Roguing for Disease Control**

Since the 1920s, roguing to remove and destroy infected cane plants has been one of the main methods of controlling Fiji disease and downy mildew in Fiji. Its main objective is to reduce the initial inoculum and decrease the rate of disease development. Each mill area has a roguing gang whose daily task is to inspect cane stools row by row and rogue out diseased plants. However, this roguing has two limitations. Firstly, stools that are infected but show no symptoms cannot be detected. Secondly, roguing will succeed only where disease incidence is low and cannot really cope in areas where very susceptible varieties are planted. Currently, with Mana as the major cane variety in Viti Levu, the existing roguing system is able to keep both major diseases under satisfactory control.

### **Sugarcane Germplasm and Quarantine**

The Fiji Sugar Corporation germplasm collection is planted in three locations on Viti Levu — Dobuilevu (intermediate zone), Drasa (dry zone) and Lautoka (dry zone). It consists of noble, wild and hybrid canes. A total of 3300–4500 varieties are maintained in each site. Since 1973 about 1300 of these varieties have been imported from countries such as Australia, France, Guyana, Hawaii, India, Indonesia, Jamaica, Papua New Guinea, the Philippines, Réunion, South Africa, Thailand and the United States of America. The majority of the germplasm collection are local hybrids. The germplasm is used mainly for sources of flowers in crossing and breeding work, but also for variety collection and exchange with other countries.

From 1973 to 1988 all imported germplasm varieties were placed in Beltsville, Maryland, USA, for close quarantine for one year before being brought to Fiji. Once these varieties arrived in Fiji, they were grown in open quarantine for at least a year before they could be included in the germplasm collection. The main purpose of open quarantine is to check whether any diseases, foreign to Fiji, have been brought in with the shipments of cane setts, and to destroy any plants developing disease.

Prior to 1973 the island of Malolo Lailai was used for open quarantine. After the discovery of smut in Hawaii in 1971, all Hawaiian and Australian varieties at Malolo Lailai were destroyed. As an additional precaution, it was recommended that all Hawaiian varieties be grown for one year in the Colonial Sugar Refining Company (CSR) Sydney quarantine, before being introduced to Fiji. In 1973, because of water



shortages, the open quarantine station was shifted to Yaqara Cattle Ranch on Viti Levu. However, in 1988 sugarcane mosaic virus was found in Yaqara on two cane varieties introduced from the Philippines. All of these canes were destroyed and the Fiji Sugar Corporation did not import any more overseas varieties for the next six years. But after considering cane breeding needs, imports of new varieties started again in 1995, beginning with five varieties from Brazil. At present the Fiji Sugar Corporation is using the Ministry of Agriculture, Fisheries and Forestry (MAFF) Koronivia Post Entry Quarantine Station as closed quarantine and Nausori Highlands as open quarantine.

### Conclusions and Recommendations

Imports of sugarcane germplasm from the 1870s to the 1940s, before the establishment of plant quarantine, may have brought in some of the diseases that are now found in Fiji. The risks of introducing new diseases, especially viral, due to increasing germplasm exchange now and in the future, poses real hazards for sugarcane production in Fiji. However, the benefits of using germplasm materials for breeding purposes may outweigh those risks.

To lessen the risks of introducing new diseases through imports of sugarcane germplasm, the following action plans are recommended for Fiji.

#### National level

1. Train the crop protection staff (scientific officers and technicians) of the Fiji Sugar Corporation in appropriate techniques for diagnosing disease in both field (including germplasm collections) and quarantine (open and closed) situations.
2. Train quarantine officers in sugarcane disease detection and rapid diagnosis of germplasm materials.
3. Train scientific officers of the Fiji Sugar Corporation in quarantine principles and regulations.
4. Link workshops between quarantine and scientific officers to exchange information and develop strategies for minimising risks.
5. Develop public awareness campaigns that are aimed at sugarcane growers and the general public.

#### International level

1. Enforce international technical guidelines regarding the safe movement of sugarcane germplasm, such as the FAO/IBPGR guidelines (Frison and Putter 1993).

2. Establish an intermediate quarantine station outside Fiji to screen viral and other unknown diseases.
3. Implement active international research collaboration on important issues that can lessen the disease risks involved with exchange, maintenance and conservation of sugarcane germplasm.

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# Major Diseases Affecting Sugarcane Production in Australia and Recent Experiences with Sugarcane Diseases in Quarantine

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## Abstract

Ratoon stunting disease causes the greatest direct economic losses of any sugarcane disease in Australia. Losses are estimated at between A\$10 million and A\$20 million a year in spite of control programs that cost in excess of A\$2 million a year. Fiji disease threatened the existence of the sugar industry in southern Queensland during the 1970s. Since the replacement of the susceptible variety NCo310 with resistant varieties, Fiji disease has been successfully controlled but it is still responsible for restricting the use of potentially productive germplasm. Other important sugarcane diseases in Australia include leaf scald, mosaic, *Pachymetra* root rot, red rot, rust, yellow spot and pineapple disease. Two minor virus diseases unique to Australia are striate mosaic and dwarf.

Recently sugarcane mosaic, sugarcane bacilliform virus and yellow leaf syndrome have been detected in imported germplasm. Noble canes recently imported from India developed severe yellow-red flecks and were subsequently destroyed.

SUGARCANE is grown in Australia from Grafton in New South Wales to Mossman in far northern Queensland. In 1994 sugarcane was harvested from 365 000 hectares, from which 5 million tonnes of raw sugar were produced. Sugar is the second largest export earning crop in Australia, with export earnings of A\$1.5 billion in 1994. The industry is expanding at about 5% a year and new production areas are being established on the Atherton Tableland of Queensland and the Ord River district of Western Australia.

The control of sugarcane diseases has played an important role in maintaining the viability of the sugar industry in Australia throughout its history. In the late 1800s gumming disease caused extensive losses and impeded the milling processes. The importance of this disease was one of the factors that lead to the establishment of the Bureau of Sugar Experiment Stations in 1900. Gumming disease has subsequently been eradicated from Australia. In the 1970s the Fiji disease epidemic in southern Queensland threatened the existence of the industry in this region.

Quarantine to prevent the introduction of diseases not present in Australia and strains of pathogens not present in Australia has enabled the Australian sugar industry to remain free of some important diseases.

Australia remains one of a few countries in which sugarcane smut has never been recorded.

## Fiji Disease

Fiji disease is caused by a virus classified as belonging to the reoviridae (Egan, Ryan and Francki 1989). Raised galls running along the vascular bundles on the undersurface of the leaf blade, midrib or leaf sheath are its diagnostic symptom. Fiji disease is spread by plant hoppers of the genus *Perkinsiella* including *P. vastatrix* Breddin and *P. saccharicida* Kirk.

The devastation and losses caused by Fiji disease in the variety NCo310 in Bundaberg in the 1970s threatened the viability of the industry in this district. The disease spread rapidly through the district, and severely affected fields produced no millable cane. The highly resistant varieties introduced to overcome the epidemic had lower productivity, and productivity continued to be affected for ten years after the epidemic subsided until higher yielding resistant varieties were bred (Cox and Hansen 1995). Fiji disease was recorded for the first time in central Queensland in 1980 and resulted in the replacement of NCo310 by more resistant varieties in this district. Fiji disease has never been recorded north of Proserpine in Queensland.

Resistant varieties have successfully controlled Fiji disease and no disease has been recorded in Bundaberg for a number of years. Fiji disease still occurs at a low level in fields in central and southern Queensland and northern New South Wales. Highly sensitive PCR-DNA probes have been developed for the Fiji disease virus (Smith, Van de Velde and Dale 1992).

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## Ratoon Stunting Disease

Ratoon stunting disease was first discovered in Australia in 1944-45 (Steindl 1961). The disease is caused by the xylem-limited bacterium *Clavibacter xyli* ssp. *xyli*. The bacterium restricts water movement in the xylem and causes yield losses of 5-50%, depending on varietal susceptibility and environmental conditions. Examination of the xylem contents for the characteristic bacterium with a phase-contrast microscope is widely used in Australia for diagnosis (Steindl 1976), and in recent years enzyme immunoassays (Croft et al. 1994) and fluorescent antibody staining (Davis and Dean 1984) have been used for diagnosis. PCR-DNA probes have been developed and promise to offer greater sensitivity for detecting the bacterium (Fegan, personal communication).

Ratoon stunting disease occurs in all districts of the Australian sugar industry, with the incidence of the disease ranging from less than 1% to 50% of fields. Losses from the disease are estimated to be between A\$10 million and A\$20 million a year. But because of the difficulty in diagnosing the disease, losses could be underestimated. Ratoon stunting disease is the most economically important disease in Australia.

Control programs estimated to cost in excess of A\$2 million a year are established in all Australian sugar districts. These include hot water treatment (50°C for three hours) to establish disease-free planting material, multiplication of the disease-free cane in nursery plots and hygiene of cutting implements, including planters and harvesters.

## Leaf Scald

Leaf scald caused by the bacterium *Xanthomonas albilineans* Dowson is endemic in nearly all cane growing districts of Australia, occurring both in the cane and in alternative grass hosts (Ricaud and Ryan 1989). The main effect of leaf scald in Australia is the loss of potential clones in the breeding program where up to 20% of clones are discarded because of susceptibility each year. The disease has not caused significant yield losses in Australia for many years because of the use of resistant varieties.

## Chlorotic Streak

Chlorotic streak is a water-borne disease of undetermined etiology (Egan 1989). The disease is widespread in Australia in areas subject to flooding. It is estimated that over 50 000 hectares are subject to chlorotic streak infection in Queensland. Yield losses have been estimated at between 10% and 30% in experimental plots, and in commercial fields a 1% yield loss for every 5% increment in the incidence of the disease was estimated.

## Sugarcane Mosaic

Sugarcane mosaic caused by the sugarcane mosaic potyvirus (SCMV) has occurred in all districts of the Australian sugar industry but is rare north of Townsville in Queensland. The only strain of SCMV present in Australia is the A strain, which is considered a mild strain compared with other SCMV strains and strains of the closely related sorghum mosaic virus.

Epidemics of sugarcane mosaic occurred in the Childers region in the 1980s due to plantings of highly susceptible varieties and environmental conditions suitable for the aphid vectors. In southern Queensland a number of highly susceptible varieties have become popular in recent years and there is an increased chance of an epidemic occurring in the future.

## Common Rust and Yellow Spot

Common rust (caused by *Puccinia melanocephala* H & P Syd.) and yellow spot (caused by *Mycovellosiella koepkei* [Krüger] Deighton) are foliar diseases that can cause losses in susceptible varieties. Moderately susceptible varieties are still grown in Australia, and rust and yellow spot will cause losses in these varieties, particularly in years highly favourable to disease development.

## Pachymetra Root Rot

Pachymetra root rot was identified in the early 1980s and appears to be unique to Australia (Croft and Magarey 1989). It is caused by the oomycete *Pachymetra chaunorhiza* Croft and Dick. The oogonia of the fungus have distinctive verrucose projections and are 30-60 µm in diameter. The disease causes a soft flaccid rot of the primary roots and, in highly susceptible varieties, yield losses of 30-40% have been recorded. The disease occurs in most districts of Queensland. Varietal resistance is the only recommended control and highly susceptible varieties are not released in districts where the disease is severe.

## Pineapple Disease and Red Rot

Pineapple disease (caused by *Ceratocystis paradoxa* Moreau) rots the stalk cuttings used to propagate sugarcane and is characterised by the distinctive fruity smell of the rotting tissue. The disease is controlled by spraying the cut ends of the stalk pieces with fungicide at planting. Control failures are associated with damaged stalk pieces and the incorrect application of the fungicide. Each year A\$1 million is spent on fungicides to control pineapple disease.

Red rot (caused by *Glomerella tucumanensis* Arx and Mueller) can cause extensive yield losses including reduced sugar content when standing stalks become infected. In the 1994 crop in northern New

South Wales red rot severely affected yields in the popular cultivar, Dart. Isolated cases of severe yield loss have occurred in southern Queensland and in the Burdekin district in susceptible cultivars.

### **Striate Mosaic and Dwarf Disease**

Striate mosaic is a virus disease that causes fine striations in the leaves of infected plants, severe stunting and ratoon failure. The disease has been recorded only in the Burdekin district of Queensland and occurs only on isolated patches within fields. The infested patches do not appear to spread except when diseased planting material is planted into new areas. Even then there is no record of the disease establishing in new areas from diseased planting material. In pot experiments soil from these sites has transmitted the disease. No virus particles have been observed in infected leaves but dsRNA has been isolated and is currently being cloned so that specific probes can be developed (Choi and Randles, personal communication).

Dwarf disease is a probable virus disease that has been recorded in central and southern Queensland. It causes severe stunting, stiff erect leaves giving a fan appearance, and fine white stripes on the leaves. Sections through the white stripes show distorted vascular bundles and lignification of the phloem. The disease has occurred in isolated areas that have never previously grown sugarcane, suggesting that a native host is involved. No viral particles have been found and the method of transmission is unknown. The disease occurs sporadically and has never caused serious yield losses, but its presence in isolated nursery plots has caused concern.

### **Recent Experiences with Germplasm Imported into Australia**

#### **Sugarcane mosaic virus**

In 1989 the sugarcane mosaic virus was intercepted in quarantine at the Bureau of Sugar Experiment Stations (BSES), Brisbane, in variety C-GD19 imported from China earlier that year. The sugarcane clone showed the typical light green mosaic pattern of SCMV infection, and the causal agent was confirmed by Dr D.S. Teakle following electron microscopy of leaf dips. The strain of SCMV was not determined and the sugarcane clone was destroyed. Other C-GD clones in the shipment did not develop symptoms of mosaic infection or any other disease and were released in 1990.

#### **Sugarcane bacilliform virus**

Sugarcane bacilliform virus (SCBV) was first reported as a sugarcane pathogen by Rodriguez Lema et al. (1985), and was described in detail by Lockhart and Autrey (1988). In 1992 BSES imposed a moratorium on the release of SCBV-infected sugarcane clones from

quarantine as more information became available and concerns grew about SCBV and the closely related banana streak virus (BSV). The three major issues were:

- there was little information about the effect of this pathogen on sugarcane yield;
- there was information from overseas indicating differences in aggressiveness of different isolates of SCMV and BSV; and
- some strains were not detected by the current tests.

In 1992 two clones were identified as SCBV infected by immunosorbent electron microscopy by Dr D.S. Teakle and colleagues and were not released. In 1993 better antisera supplied by Dr B.E.L. Lockhart and a PCR-based test still being developed at BSES were used to screen the quarantined clones. Twenty-four clones (67% of the foreign clones) were found to be infected. In 1994 all quarantined clones were destroyed due to symptoms of yellow leaf syndrome in some foreign clones, and the extent of SCBV infection of the collection was not determined.

#### **Sugarcane mild mosaic virus**

Sugarcane mild mosaic virus (SCMMV) was not reported to be present in Australia until Dr Lockhart demonstrated its presence in 1994. Symptoms of SCMMV infection include yellowish striations with indistinct edges in the leaf blade. SCMMV symptoms have not been observed in *Saccharum* hybrids, but have been noted in *Saccharum officinarum* clones. SCMMV is often found as a mixed infection with SCBV, and only recently has a good antiserum been available to assay for SCMMV.

#### **Yellow-red leaf fleck**

In 1994, 200 *S. officinarum* clones were imported from the Indian world collection of *Saccharum* germplasm and placed in quarantine in January 1995. Symptoms of SCBV (leaf flecks) and SCMMV (chlorotic striations) were soon evident in many clones in this collection. In May a new type of symptom was observed. Yellow and red leaf flecks appeared on the leaf laminae of a large number of the clones. These flecks expanded and eventually covered a significant area of the leaf. Inspections over time suggested that the yellow-white chlorotic flecks appeared first and progressed to red-brown necrotic areas. There were differences in the symptoms of the sugarcane clones. In some clones one of the symptoms predominated, while in others both symptoms were equally apparent. These symptoms were somewhat similar to red leaf mottle caused by the peanut clump virus of commercial canes. Samples sent to France to test this hypothesis were negative by enzyme-linked immunosorbent assay for peanut clump virus when tested by Dr M. Chatenet. However, the antiserum used was raised against an African isolate of peanut clump virus, and the Indian peanut clump virus is

reported to be serologically different. The entire collection was destroyed and the cause of the yellow-red leaf fleck remains undetermined.

### Yellow leaf syndrome

Some of the first reports of yellow leaf syndrome were published in early 1994 (Borth, Hu and Schenck 1994; Comstock, Irvine and Miller 1994) and later that year yellow leaf symptoms were observed in quarantined cane. The major symptom of a yellow midrib (that may have some red flecking) and a green leaf blade was evident in a number of quarantined clones. The causal agent was suspected to be a closterovirus (Borth et al. 1994) and, as yellow leaf symptoms were present in a number of clones, the decision was made to destroy all quarantined canes. Subsequently symptoms similar to yellow leaf syndrome were observed in clones from Brazil, East Africa, Hawaii, the Philippines, Réunion, Malaysia, Taiwan and Cuba and in Australian bred clones on BSES experiment stations. Research to identify the causal agent of yellow leaf syndrome are continuing in Brazil, the United States and Australia.

### Results of Recent Quarantine Experiences

Quarantine is the process and infrastructure to provide and maintain access to foreign germplasm for breeding and research. Recent events have seriously interrupted access to this important germplasm, with some important breeding programs postponed or restricted. BSES, in collaboration with university scientists and with support from the Sugar Research and Development Corporation, has initiated research into SCBV and unidentified diseases such as yellow leaf syndrome. In the short term, until the causal agents of new diseases are identified and there is better understanding of new pathogens such as SCBV and SCMMV, there will continue to be a need to destroy infected germplasm to protect the sugar industry.

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# Sugarcane Diseases in Southern Brazil: A Brief Report

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## Abstract

The principal diseases in sugarcane in southern Brazil are smut (*Ustilago scitamineae*), rust (*Puccinia melanocephala*), ratoon stunting (*Clavibacter xyli*), leaf scald (*Xanthomonas albilineans*), mosaic and yellow leaf syndrome.

THE MAJOR DISEASES affecting sugarcane in southern Brazil are smut, rust, ratoon stunting, leaf scald, mosaic and now yellow leaf syndrome. Sugarcane occupies 2.2 million hectares of the state of São Paulo (45% of the sugarcane area in Brazil). The major commercial varieties and their reaction to diseases are shown in Table 1.

## Smut (*Ustilago scitaminea*)

A large survey over a wide area in 1991 of the major commercial sugarcane varieties showed that plant cane of SP71-6163 and SP71-1406 presented an average of 500 infected stools per hectare. Experiments at Copersucar indicate that the yield loss due to smut is on the average 0.78% for each 300 stools infected per hectare.

A greater importance was given to smut in the past (5–10 years ago) and it was the reason for eliminating ten years ago the major commercial variety (NA56-79), which occupied 46% of the area cultivated with sugarcane in 1983. SP71-6163, SP71-1406 and SP79-1011 have NA56-79 as a parent and carry some of its susceptibility.

Smut is routinely inoculated in clones in the breeding program. Inoculation consists of dipping three eye sugarcane setts for 15 minutes in a suspension of smut spores (1 g of 80% viable spores per litre of water). In the breeding program, in intermediate stages of selection, approximately 50% of the clones found to be resistant to smut score 1, 2 or 3.

## Rust (*Puccinia melanocephala*)

Rust was first observed in Brazil in 1986. Breeding programs have not yet adapted entirely to the rust situation and susceptible varieties are still widely cultivated. Some rust susceptibility is accepted in varieties to be released, due to the lack of better options.

Experiments conducted at Copersucar show that yield losses on susceptible varieties can be of the order of 47%.

Rust seems to be more aggressive today than it was five years ago, and some previously intermediate varieties are showing more susceptible reactions. This is possibly due to a greater inoculum and not necessarily to a more aggressive pathogen.

There is a feeling among growers and some breeders that it is possible to cultivate rust susceptible varieties by planting and harvesting at appropriate times. Rust seems to affect crops more when they are between three and five months old. Crops older than this exhibit much less rust. Therefore crops will be less affected if harvested or planted so that they are older than five months in September or May, after which the spore load in the air begins to rise (Figure 1).

Copersucar inoculates young sugarcane seedlings with rust for heritability studies. Sixty-day-old seedlings are placed in flats and covered with a thatched roof of sugarcane leaves with a heavy rust spore load. After 20 days in these conditions, individual plants are scored for rust reaction on the leaf number +1. Heritability values of 75% were obtained in a parent X offspring regression experiment, with 51 full sib and 144 half sib families using 90 seedlings per family.

Rust testing in clones depends on natural infection in trials that are bordered with susceptible varieties. In the breeding program, in intermediate stages of selection approximately 60% of the clones found to be resistant to rust score 1, 2 or 3.

Rust reaction is considered a potential candidate for molecular marker studies. Unselected progenies, segregated for rust reaction, are being kept in the field. Work with tissue culture has shown that it is not uncommon to find a variant with a different rust reaction from the donor parent, usually a more susceptible reaction. Susceptible clones have even been found in field-grown plants of resistant varieties. These plants are being screened with molecular markers.

## Ratoon Stunting Disease (*Clavibacter xyli*)

Copersucar has established a 'dot-blot' based diagnostic as a service to mills. A minimum of 100 samples are

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**Table 1.** Distribution of major commercial varieties of sugarcane in southern Brazil and their reaction to diseases (disease scores on a 1 to 9 scale).

Variety	% of area <sup>a</sup>	Smut	Rust	Mosaic	Leaf scald	Ratoon stunting <sup>b</sup>	Yellow leaf <sup>b</sup>
SP71-6163	22.6	5	6	2	2	-	++
SP70-1143	21.9	2	6	2	6	+	-
RB72454	16.8	6	1	2		+	+
SP71-1406	10.8	6	7	2	2	+	+
SP79-1011	5.6	4	7	2	2	+	+
RB765418	4.8	4	1	2		+	+

<sup>a</sup> Of 2.2 million hectares. <sup>b</sup> ++ = severe symptoms; + = symptoms observed; - = no symptoms observed.

taken from each area to be diagnosed. Samples consist of approximately 0.5 mL of xylem fluid extracted from a basal node, using positive air pressure. Samples are extracted in the field into microfuge tubes. A drop of 5% formaldehyde solution is added to the tube as a preservative.

Samples are received at Copersucar and 0.3 mL are placed on nitrocellulose membranes (100 + 4 controls on a 12 by 7.5 cm membrane). The diagnostic is based on polyclonal antibodies produced in rabbits. The system detects concentrations of 10<sup>4</sup> bacteria per millilitre.

The six major commercial varieties accounted for 208 areas analysed (20 800 samples) in 1993-94 and 286 areas (28 600 samples) in 1994-95. The average infection of these varieties was 0.75% in 1993-94 and 4.1% in 1994-95. The number of areas tested positive for ratoon stunting disease (at least one sample posi-

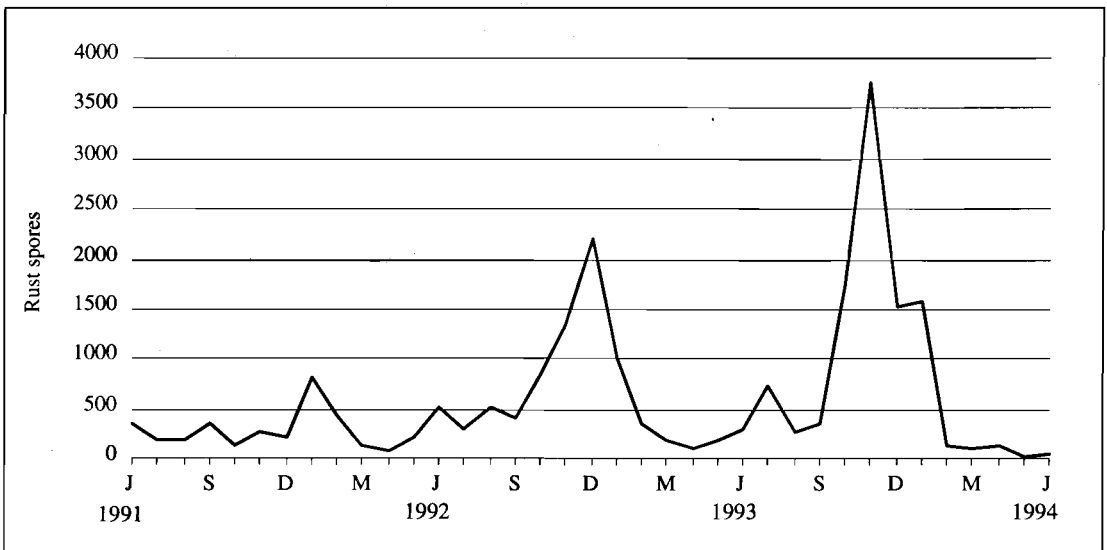
tive) was 21% in 1993-94 and 39% in 1994-95. It should be pointed out that these data refer to cane that is to be used as seed.

Although there is genetic variability for ratoon stunting disease reaction, Copersucar has not quantified this variability and has not initiated breeding for resistance.

### Mosaic

Most sugarcane varieties are resistant to the mosaic strain B, the prevalent strain in the field, and for that reason mosaic is not seen by growers as a problem. The breeding program inoculates seedlings from crosses using susceptible parents, and discards susceptible plants at an early stage — approximately 20 000 seedlings of the 600 000 seedling program.

Mosaic is commonly intercepted in quarantine, based on inoculation in Sorghum Rio. The most recent



**Figure 1.** *Puccinia melanocephala* spores collected on a 2.5 cm<sup>2</sup> glass slide at the Copersucar Technology Center from 1991 to 1994.

interceptions were three Thai varieties (K80-1-128, K81-1-026 and K84-69).

In seedlings mosaic is inoculated with an air gun and carborundum, while in clonal evaluations Copersucar relies on natural infection from susceptible varieties planted in alternate rows. In the breeding program, in intermediate stages of selection, approximately 80% of the clones found to be resistant to mosaic score 1, 2 or 3.

### Leaf Scald Disease (*Xanthomonas albilineans*)

Leaf scald disease has grown in importance in recent years. Some major commercial varieties have tested susceptible to the disease, but the greatest worry is with some very promising new clones that have shown unacceptable reactions. With the increasing mechanisation of harvesting in Brazil, more importance should be given to leaf scald disease resistance.

Various isolates of *X. albilineans* were sent to Montpellier and were identified by P. Baudin as being of serotype 2. Copersucar has produced a polyclonal antibody on rabbits that has shown the same sensitivity as antiserum samples obtained from Montpellier.

Variety reaction is evaluated in artificially inoculated plants. One eyed sets are vacuum inoculated with 0.2 mL of diluted *X. albilineans* suspension. In the breeding program, in intermediate stages of selection, approximately 40% of the clones found to be resistant to leaf scald disease score 1, 2 or 3.

### Yellow Leaf Syndrome

Yellow leaf syndrome was observed in some areas in the southern part of the State in 1991-92. One variety in particular, SP71-6163, showed symptoms of midrib yellowing and stunting of growth. The symptoms worsened in 1992-93 and began affecting yields of this

variety on a state-wide basis. Initial studies considering both biotic and abiotic hypotheses included soil borne fungal pathogens, nutrition, soil compaction and climatic conditions, among others.

Although some abiotic factors such as nutrition and soil compaction appeared to enhance symptoms they could not be regarded as the sole causes of the problem. Poor rooting in plants with symptoms was suggestive of soil-borne pathogens but these were ruled out as the causal agents after extensive, unsuccessful attempts to isolate soil pathogens.

Similar symptoms were observed in Hawaii, and Susan Schenck reported the isolation of double stranded RNA. The RNA isolation was repeated at Copersucar. In some cases Copersucar was able to isolate RNA from clones with symptoms. Microscopic investigation of sugarcane tissue revealed abnormalities in phloem tissue. In fresh samples autofluorescence was observed in the phloem. Necrosis in the phloem was also observed. However, the most fluorescent cells are not the necrotic ones. It is possible that necrosis is the final stage, which is preceded by the accumulation of fluorescent material (phenyl-propanoids?) as a reaction to viral infection. A 'tissue blotting' technique was employed to imprint a leaf sample on nitrocellulose membrane. The blots were challenged with BYDV-PAV antiserum obtained from Cornell University. A positive reaction was observed in the phloem in some of the samples analysed.

Electron microscopy has proved to be difficult due to the toughness of the leaf samples. In the cytoplasm of some phloem companion cells of plants with symptoms, groups of isometric particles of 20-25 nm in diameter were observed. These fit the description and size of luteovirus barley yellow dwarf virus. Transmission tests with aphids are being conducted to confirm the luteovirus as the causal agent of yellow leaf syndrome.



# **Excluding Diseases in Exchange of Vegetatively Propagated Crops**

# Optimising Disease Expression in Quarantine

R.L. Dodman\*

## *Abstract*

Environmental conditions affect all stages of pathogen development, including symptom expression. Post-entry quarantine of imported germplasm relies heavily on the expression of disease symptoms to detect lines of germplasm that are carrying diseases that could be of quarantine concern. Providing post-entry conditions that facilitate the detection of diseased germplasm is an important aspect of minimising the risk of introducing exotic diseases with new germplasm.

THE RELATIONSHIP between the host, pathogen and environment is fundamental to plant pathology. The disease triangle featuring these key elements is discussed at length in all basic plant pathology texts (see, for example, Agrios 1978 and Matthews 1970).

In examining pathogen life cycles, all parts of the disease cycle have been investigated and the role of the environment in each phase studied in detail. The main environmental factors — water (that is, rain, dew and humidity), temperature and light — have a role in the dispersal and deposition of pathogen propagules, in the germination of these propagules, in the penetration of the host and in the subsequent development of the pathogen within the host, leading to reproduction and the commencement of the next cycle with further dispersal.

For quarantine, the most important part of this disease cycle and interaction with the environment is the final phase — the development of the pathogen in the host and the expression of symptoms. However, some other parts of the disease cycle are relevant with some pathogens (particularly bacteria) and will be discussed in more detail later.

## **Post-Entry Quarantine**

Each country has conditions for imported material. For germplasm these conditions vary depending on whether the material to be moved is seed or vegetatively propagated material. This general discussion covers all types of germplasm.

The post-entry quarantine process allows for a period of plant growth during which it is expected that any disease present will be expressed. Thus diseased germplasm will be identified and either destroyed or freed of the pathogen (if possible). This process is designed to prevent the introduction of quarantinable pathogens.

By far the greatest amount of material entering Australia comes in as seed. If restrictions apply, the seed is planted in post-entry quarantine (in some cases after specified treatments) and the plants are grown to maturity. If no disease is detected during growth the seed produced from these plants is released; the original seed is not released from quarantine. If any disease symptoms are observed, the plants in that line may be destroyed after consultation with the Chief Quarantine Officer (Plants).

There is an extremely important difference with vegetative material — a difference that hardly needs pointing out to those moving sugarcane germplasm. The difference is that it is the actual material that is introduced (not its progeny) that is eventually released after a specified period in quarantine if no disease is detected. One of the significant consequences of this is that there is the potential for the complete range of diseases associated with vegetative material to be imported whereas there is a much smaller suite of diseases associated with seed (because seed usually carries only a small proportion of plant diseases).

The two main ways of detecting disease in post-entry quarantine are by the expression of disease symptoms and by active screening (for example, virus detection with the electron microscope, transmission to indicator hosts, and probes). Most material passing through post-entry quarantine is screened on the basis of symptoms alone and thus great reliance and importance are placed on disease expression. Because of this, the relationship between environmental conditions and disease expression in post-entry quarantine is of considerable importance.

The main classes of disease (fungal, bacterial and viral) and some of the environmental factors that could be of importance for disease expression are now considered.

## **Fungal Diseases**

In the detection of fungal diseases in post-entry quarantine, the important part of the disease cycle is symptom expression and, in general, environmental factors

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have relatively little effect on this. As a consequence there is no particular emphasis on optimising environmental conditions for the expression of fungal diseases in post-entry quarantine. In general, plants develop under whatever conditions prevail in post-entry quarantine. Whether it is because of this or despite it, the expression of fungal diseases (of exotic origin, anyway) is fairly rare. Occasional incidences of *Colletotrichum* leaf spots have occurred in some low risk ornamental plants, but have rarely been considered of importance.

A combination of factors — seed treatment, low level of seed transmission, low level of successful establishment from inoculum in or on seed, low level of invasion of seed on a diseased plant and general high health of seed material being imported — reduces the likelihood of disease being introduced and becoming established in plants in post-entry quarantine. The introduction of seed through post-entry quarantine is thus a very effective way of eliminating fungal diseases.

The risks are much greater with vegetatively propagated material. Systemic infection (for example, downy mildew) and latent infections are always possible, and post-entry quarantine conditions (both the time in post-entry quarantine and the environment) should ensure that symptoms are expressed in infected material. Various factors (for example, bare-rooted dormant cuttings and heat treatment and/or dipping of the material) are used to minimise risks. However, environmental manipulation is rarely employed. Rather the period in quarantine is usually set so that the material must grow through a whole season (or more) to provide a range of environments where disease can be expressed.

### Bacterial Diseases

There are some significant differences between fungal and bacterial diseases that are important in the quarantine situation. Perhaps the most important is the potential for bacterial pathogens to develop epiphytically on the host without actually infecting and producing symptoms. It is then possible for bacteria to be present on the seed that is produced or to remain in this epiphytic state on the leaves until the vegetative material is released. Pathogens can thus escape detection in post-entry quarantine and subsequently establish in the field. More measures for protecting against this pathway for breaching quarantine (such as more sensitive detection procedures) need to be considered.

Because environmental conditions, particularly the presence of water, are so important for bacteria to gain entry to their host, there are situations where the conditions of entry specify that leaves should be kept wet during initial growth to encourage disease expression. This occurs with cotton where seedlings must be

grown under continuous mist for 21 days at temperatures above 25°C so that conditions for infection by *Xanthomonas campestris* pv. *malvacearum* carried in seed are optimised. During later stages of growth, plants are sprayed twice with copper oxychloride to minimise the opportunities for epiphytic growth on leaves and the transmission of bacteria to developing seed.

The value of optimising conditions to encourage disease compared with providing conditions that restrict bacterial development can be debated. The issue is certainly important, particularly for vegetative material where a resident epiphytic population could be very significant. This issue is also relevant for some vegetative material that is introduced in tissue culture. There is the belief that bacteria in tissue culture will be detected on the medium, but it is also argued that bacteria could grow epiphytically and never develop on the medium. This is another important issue to be resolved and where sensitive detection procedures could be of value.

### Virus Diseases

Environmental factors can have profound effects on the course of virus infection and subsequent disease and symptom development. This has important implications for material being screened and also for indexing procedures used during post-entry quarantine.

The important processes influenced by the environment are infection, virus multiplication in the host and the development of symptoms. The most important factors are temperature, water, light and plant nutrition.

Maximising virus multiplication and usually the intensity of virus symptoms is usually favoured by growing plants that are properly watered and have adequate light and ventilation and a good balance of all the essential nutrients. In general, it is best to keep temperatures below 30°C as there is evidence that the symptoms of some viruses in new growth may be milder or even totally masked when temperatures are excessive.

In general, similar conditions favour virus infection although there is evidence that a period of low light intensity or even darkness during the day before inoculation can increase the susceptibility of the host. There are also observations that in some cases a period of high temperature before inoculation can lead to greater infection. Another observation is that more disease may develop with inoculations in the afternoon than in the morning. These observations are important for indexing procedures carried out in post-entry quarantine.

From this information it can be seen that reasonable control of environmental conditions during post-entry quarantine is highly desirable if the detection of virus symptoms is to be used as a reliable indicator of germplasm health.

## **Current Post-entry Quarantine Practices for Sugarcane in Australia**

The conditions that apply for the importation of sugarcane germplasm into Australia provide three options involving different choices of hot water treatment and a period of growth in post-entry quarantine. The current regime used by the Bureau of Sugar Experiment Stations in Australia is even more stringent than that specified by the Australian Quarantine and Inspection Service. Although the treatment used will effectively eliminate downy mildew and smut, several virus and some bacterial diseases could still be present after the treatment. Reliance on the treatment and on symptom development during growth in post-entry quarantine has been an effective means of detecting exotic diseases in the past. Currently the conditions for sugarcane germplasm entry into Australia are under review, the aim being to develop a set of conditions that will be practical (that is, allow the ready introduction of

germplasm) but will provide adequate safeguards for the Australian sugar industry.

In summary, the post-entry quarantine process that has been and still is being used has been effective without particular control of environmental conditions. A range of environments over the period in quarantine has generally allowed expression of disease symptoms. However, there is concern that some diseases, particularly some virus diseases, could escape detection because symptoms are not produced. Some form of indexing with appropriate detection procedures may need to be introduced to ensure that such diseases are found during the exchange of germplasm.

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# Role of In-Vitro Maintenance of Sugarcane for Germplasm Conservation and Exchange

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## Abstract

The biology of sugarcane makes it useful to develop in-vitro germplasm conservation and exchange methods. Various approaches exist, using somatic embryogenesis or bud and meristem culture. Bud and meristem culture from disease indexed materials provides a suitable source for producing in-vitro plantlets that can be exchanged and quickly multiplied through micropropagation. It may help eliminate diseases of unknown etiology. Experience with medium term conservation of in-vitro plantlets in slow growth conditions illustrates its vulnerability; duplication of such collections is necessary if they are to ensure the conservation of unique materials. Medium or long term conservation may, however, be better ensured by apex cryopreservation. Existence of such source material for in-vitro plantlets regeneration, exchange and multiplication will help keep the active in-vitro phases — and the genetic instability possibly associated — to a minimum. For those resources that are to serve only as sources of genes for breeding, somatic embryogenesis offers potential additional options that have to be explored according to the community needs and the constraints of sexual seed production, conservation and exchange.

THE MAINTENANCE COSTS of large sugarcane field collections are high and plants in natural conditions remain exposed to pests and pathogens as well as to natural disasters. Seed conservation is possible but it is a very destructuring process for this highly heterozygous plant. Moreover, the flowering ability is low for many clones. In-vitro culture techniques allow conservation of plant germplasm in a pathogen-free condition with reduced space requirements and maintenance costs (Engelmann 1991). For short or medium term storage, the aim is to reduce the growth rate of the plant material, usually by lowering the culture temperature. For long term conservation, cryopreservation (liquid nitrogen,  $-196^{\circ}\text{C}$ ) is preferred since it stops all metabolic events and cell divisions. The plant material can thus be stored for extended periods without genetic alteration. While cultures stored under slow growth are the source of material for exchange between genebanks and for distribution to users, material stored under cryopreservation is conserved for the long term in safe conditions and with limited maintenance.

## Current Applications of In-Vitro Culture for Sugarcane Germplasm Management

The application of in-vitro culture to sugarcane has been reviewed by Maretzki (1987) and by Guiderdoni et al. (1995) for somatic embryogenesis.

## For large scale propagation

Research on somatic embryogenesis in sugarcane was directed mainly toward the production of useful somaclonal variants and, more recently, genetic transformation. Even though significant progress has been made, somatic embryogenesis is still far from being usable for the large scale propagation of sugarcane.

Propagation techniques for sugarcane using meristem or bud culture have very high multiplication potential since it is theoretically possible to produce millions of plants per year from a single apex. This methodology is being applied in various scales in several countries for the rapid multiplication of new varieties.

## For disease eradication and germplasm exchange

In-vitro culture of apices has been extensively used for producing disease-free material, sometimes in conjunction with heat therapy, and was proven to substantially eliminate various pathogens. This method is recommended by the Food and Agriculture Organisation and the International Board for Plant Genetic Resources for safely moving sugarcane germplasm, since it is the most effective in eliminating most fungal and bacterial diseases and may currently be the only option to eliminate diseases of unknown etiology. In-vitro culture of apices is now routinely used for the international exchange of disease-free plant material for this crop (de Boer and Rao 1991; Paulet and Glaszmann 1994).

## For medium term conservation of germplasm

Experiments performed in India demonstrated that rooted plantlets could be stored at  $25^{\circ}\text{C}$  for one year with one subculture after six months (Srinivasan and Srinivasan 1985). In Australia, 200 *Saccharum* spp.

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hybrid clones were stored for twelve months at 18°C and no phenotypic modifications were noted in the recovered plantlets (Taylor and Dukic 1993).

Extensive experience was acquired on the routine use of in-vitro culture on a large scale for conserving sugarcane at CIRAD, Montpellier (Paulet and Glaszmann 1994). The in-vitro collection of CIRAD was established in 1981, in connection with the International Quarantine Center for sugarcane hosted by this centre.

#### *Technique*

The procedures for the in-vitro inoculation and conservation of sugarcane germplasm are the following. In-vitro cultures are initiated using axillary buds sampled on canes that have been submitted to two successive quarantine cycles, and can thus be considered disease-free. The plantlets originating from bud development are then multiplied until a sufficient number is available. For storage, plants are placed at 18°C on a diluted MS medium under reduced light intensity. Under these conditions, subculture intervals vary between six months and two years, depending on the variety. Between two storage periods, plants are placed for 2–4 weeks at 25°C to stimulate root initiation. When a given variety is requested, plantlets are transferred at 25°C for multiplication. The plant material is dispatched in the form of in-vitro rooted plantlets 3–6 months after the request is made, depending on the quantity of material to be produced.

#### *Management aids*

The plant material of the in-vitro collection has been partly characterised using isoenzymatic and, more recently, RFLP (restriction fragment length polymorphism) profiles (Eksomtramage et al. 1992; Lu et al. 1994). In addition to the precise description of the varieties included in the collection, these techniques may allow the possible modifications occurring in the in-vitro material over extended storage periods to be assessed.

#### *Constraints*

The in-vitro collection has included up to 650 varieties. Some varieties have been stored under slow growth for up to 13 years. The management of the in-vitro collection requires one full time staff member. Moreover, it would be advisable to reintroduce varieties after several years of such conservation in order to minimise risks of genetic instability and drift. The size of the collection was thus decreased to about 300 clones in 1994.

#### *Safety*

In late 1994 there was a sudden boom in fungal and bacterial contamination of tubes in the 18°C room. It was several weeks before the main factor could be identified. Several mite species of the family

Pyemotidae were found to be present and getting into the glass tubes by crawling along the folds of the wrapping film (Saran wrap) and perhaps even through it. This invasion could be controlled after several mist applications of a miticide above the opened tubes. The result was a 90% loss of accessions. Although this was the first time such an accident occurred in more than ten years experience, the result illustrates that in-vitro conservation in slow growth conditions is not devoid of hazards, even in a laboratory that has broad and lengthy experience.

#### **For long term conservation of germplasm**

Cryopreservation experiments have been performed using cell suspensions, embryogenic calluses and apices of sugarcane. Given the initial investment required for establishing cell suspensions, this material seems unsuitable for conserving large numbers of genotypes. Embryogenic calluses and apices are more likely to allow large scale conservation.

#### *Embryogenic calluses*

The first success with cryopreservation of embryogenic calluses was reported by Ulrich et al. (1979) using cultures of the variety H50-7209. Various studies brought gradual improvements to the technique. The most comprehensive study was performed by Eksomtramage et al. (1992). An efficient technique was set up with embryogenic calluses of sugarcane variety Co6415. Up to 92% regrowth was obtained from cryopreserved calluses. This technique was successfully applied to calluses of ten other varieties, with, however, a variable and sometimes limited callus regrowth and regeneration rate ranging from 5% to 39%. Numerous plantlets were regenerated and could be transferred to soil and grown in the greenhouse.

#### *Apices*

Cryopreservation was recently achieved with apices sampled on in-vitro plantlets using encapsulation dehydration (Gonzalez Arnao et al. 1993; Paulet, Engelmann and Glaszmann 1993). The technique was developed with variety Co6415, yielding a cryopreserved apices survival rate of 64%. When applied to 13 additional varieties at CNIC, Cuba, and CIRAD, survival rates ranged between 14% and 91%, with an average of 61%. Direct regrowth of cryopreserved apices occurred within three days of thawing and whole plants could be regenerated within five weeks.

#### **Investigations of Genetic Stability Through Conservation**

Trueness to type of plants regenerated after cryopreservation, using isozymes and RFLPs, was tested at CIRAD in various studies that are now summarised.

### Cryopreserved calluses

Surveys of somaclones derived from frozen calluses sometimes revealed numerous modifications for isozymes and for RFLPs with both nuclear probes and mitochondrial probes. However, variation was also observed among plants derived from unfrozen calluses; thus, it was not possible to detect a specific effect of freezing. Comparison of some variant plants regenerated from the same callus suggested that sequences of distinct modifications can occur, allowing modifications to be attributed to more than a single major event.

### Cryopreserved apices

The plants regenerated from frozen apices were surveyed with isozymes and RFLPs using 17 nuclear probes covering all known sugarcane linkage groups and three mitochondrial probes, in combination with two restriction enzymes. This represented more than 200 bands in the parental variety Co6415. Several molecular types were uncovered among the apex derived plants, be they regenerated from frozen apices or unfrozen apices. This variation was not due to freezing but was pre-existing among the in-vitro plantlets that were used for producing the apices. The differences suggested the loss of genetic material — chromosome or chromosome segment — or DNA methylation, which may be reversible. This variation must have occurred during the in-vitro phase or was present among the plants initially used for bud culture.

### Plants derived from conventional field propagation

The stability through conventional field propagation was investigated through a collaborative effort involving BSES, CERF, CIRAD (Guadeloupe), Copersucar, HSPA, ISRI, SBI and WICSCBS. Old varieties, namely B46364, Chunnee, Co6415, NCo310 and POJ2878, were introduced to Montpellier from several of these breeding stations — that is, after many years of separate propagation. They were compared on the basis of RFLPs using 35 nuclear probes and restriction enzyme HindIII. After removing those clones that appeared mislabelled, each origin could be compared with the most frequent pattern. A total of 5987 bands were thus involved in the comparison and 27 bands were affected (appeared, disappeared or had a modified intensity). The modifications appeared localised in the genome. Although it is difficult to strictly quantify the genetic changes that occurred, it remains that many cases of modifications were uncovered.

### Conclusions and Prospects

In-vitro technologies are available for supplementing field and seed conservation of sugarcane. Examining information and experience available on various technical constraints, practicality for conservation and for exchanges, safety from hazards, and genetic stability led to the following recommendations.

### Conservation of specific genotypes

In-vitro propagation from cultured apices provides a suitable source for germplasm exchange, provided that the base plant materials have been subjected to standard disease indexing methods. Apex culture will help to eliminate diseases of unknown etiology. The movement of such materials can be followed by rapid micro-propagation.

Slow growth culture conditions should be viewed as a temporary state suitable for short term conservation. (Specific factors of genetic instability cannot be ruled out, and hazards are not absent.)

When considered necessary, long term conservation of a specific genotype should be undertaken immediately after the decision that this genotype should be conserved (to avoid the natural mutation rate causing a drift), using cryopreservation of apices (to avoid somaclonal variation associated with unmastered somatic embryogenesis) derived from in-vitro plantlets that have spent little time in vitro (to avoid possible instability during the in-vitro phase). This will allow regeneration of in-vitro plantlets for germplasm exchange. Attention has to be directed to setting up a proper stock management system.

### Conservation of genes

Accessions that have to be conserved as a source of genes for breeding purposes have a wider range of options, including those that involve a callus phase. Exposure to increased genetic instability risks can be compensated for by a quicker obtention of in-vitro tissues and subsequent higher multiplication rates. Given the sequence of manipulations, it is unlikely that the same favourable gene be lost in all the plants that can ultimately be regenerated. The same phytosanitary precautions must be taken as for the genotype conservation option. The practicality of this approach must be compared with that involving seed conservation, taking into account the embryogenetic ability and the flowering behaviour of the materials.

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# New Technologies for Indexing Crops for Pathogens

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## Abstract

New technologies can be defined as molecular methods for detecting specific components of pathogens in plant material. Of these, detection of antigenic proteins by immunological methods is rapid and inexpensive, whereas nucleic acid based technologies are more sensitive but more expensive. The latter techniques are suitable for non-immunogenic pathogens, such as viroids, and for applications where zero tolerance is required, such as in quarantine situations. Moreover, nucleic acid component analysis can be used for investigations of diseases of unknown etiology.

THE IDENTIFICATION of diseases and their causal agents (pathogens) is a basic aspect of plant pathology. It impinges on all aspects of disease control, such as exclusion, eradication, avoidance, amelioration and resistance. The identification of a pathogen provides the basis for determining its biology and shows where control strategies should be directed.

Improved methods for disease and pathogen recognition are constantly being sought to satisfy the demands for cheaper and more efficient plant production, and to avoid the possibility of the development of disease epidemics. With the move from wild or mixed culture to monoculture with single crop genotypes, the risk of epidemics is increased. Because epidemics are driven by both the amount of initial inoculum ( $Q$ ) and the rate of increase in newly infected plants ( $r$ ), indexing procedures that minimise  $Q$  are needed. One desirable feature of an indexing technique is high sensitivity so that not only can inoculum levels be kept below thresholds that can produce epidemics, but pathogens may even be excluded entirely from plant propagules.

This paper compares a range of recently developed indexing technologies with special reference to application, reliability, sensitivity and speed.

## Recognising Disease

The level of severity at which a disease becomes apparent usually depends on the observer. A disease is generally recognised by overt symptoms of infection such as lesions, necrosis, chlorosis, stunting or malformation. However, much disease remains undetected, and observations of reduced crop yield may be attributed to genetics or agronomy, even when a pathogen is the cause. For example, pea seed-borne mosaic virus infection reached high levels in pea breeders' lines because of a lack of indexing (Hampton, Kraft and

Muehlbauer 1993), and multiple virus infections of vegetatively propagated crops have caused often devastating infections that were recognised as important only when the viruses had been removed. Other forms of undetected disease can result from root infections or from hidden biochemical 'lesions' arising from reduced net assimilation rates, changes in starch or water use, changes in hormone levels and organelle or membrane damage. It is possible that 'invisible' losses of up to 15% can occur with many pathogens because they do not induce obvious disease, and even higher losses may result from infection reaching such a high incidence that the infected plants can be regarded as the 'norm'. Because an absence of overt disease does not necessarily indicate freedom from pathogen induced losses, it is necessary for pathologists to have techniques available for detecting each type of pathogen.

## Causal Agents

The biotic agents of disease can be broadly classified according to their relationship with their host. The extracellular pathogens include nematodes, fungi and bacteria that grow outside their host cells, but may propagate in the intercellular spaces or xylem, or produce intracellular haustoria. Intracellular pathogens include membrane bound parasites that may be entirely located in the vascular tissue (fungal endophytes, fastidious phloem-infecting bacteria, flagellate protozoa or phytoplasmas) and those that are not membrane bound and that parasitise the symplast (viruses and viroids).

Indexing for extracellular pathogens can be regarded as routine, using existing procedures of isolation, culture and microscopy, and eradication of these pathogens is usually not complex. Indexing for intracellular pathogens is complex for reasons associated with their obligate biotrophism, and eradication is much more difficult. Therefore new technologies are needed for identifying intracellular pathogens so that they can be excluded from planting material.

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## Principles of Indexing

Biological indexing relies on the transmission and amplification of disease in sensitive alternative hosts. This method tests the biological properties of pathogens, and early successes in controlling intracellular pathogens have been due to biological indexing.

Non-biological indexing is done by component analysis and depends on the recognition of components specific to the pathogen or pathogen group. Electron microscopy, immunology, nucleic acid probing and polymerase chain reaction (PCR) amplification are applications of this form of analysis. These methods provide information on the presence of an organism, but biological properties such as transmissibility and pathogenicity are not tested. The sensitivity of some tests using component analysis approaches that of biological testing. However, it can be argued that a test to show that a pathogen is absent (which is the aim of indexing) should be a biological one. An example of a combined test is with viroids, where an amplifying host may be used first to increase the viroid content of the tissue, while the probe is used to specifically identify the viroid. Component analysis is particularly appropriate when experimental transmission methods are not available (such as with monocotyledons that cannot be grafted), when incubation times are long, or when disease symptoms are not apparent.

### New Technologies Applicable to Non-Biological Indexing

Non-biological indexing includes methods that will recognise pathogens or their components. For example, the current scheme for classifying viruses to family and genus level relies on isolation and visualisation techniques to recognise particle shape, bounding membranes, and the characteristics of the nucleic acid such as type, size, strandedness, linearity or circularity (Francki et al. 1991). Classification to virus species or strain level uses immunology and nucleic acid sequence specific methods. These latter techniques are also applicable to prokaryotic and eukaryotic pathogens.

## Immunology

Applications of immunology rely on the specific binding of an epitope of the antigen with the paratope of the antibody. A wide range of methods has been developed to take advantage of this specific recognition (Van Regenmortel and Dubs 1993). Sensitivity has been enhanced by amplifying the antibody, usually by linking a label (Protein A), enzymes (ELISA, DIBA, SADI) or marker bodies (gold, latex) to the immunoglobulin. These methods are fast, specific, sensitive and can be cheap (Table 1). They normally require minimal laboratory facilities and some tests can be adapted to field use. Indirect methods have been developed to speed up multiple testing. Disadvantages include the small size of the antigen recognition site (epitopes are 5–7 amino acids in length), the difficulty of achieving sufficient purity of the antigen before immunisation, and the frequent problem of high backgrounds with amplified tests. Moreover, costs are higher if monoclonal antibodies are used instead of polyclonal antibodies.

### Nucleic acid methods

Table 1 provides a simple comparison of nucleic acid based methods with immunological methods. In principle, nucleic acid methods for diagnosis have a number of advantages.

- They target the genome of the pathogen.
- They may be designed to represent either the whole genome or selected motifs in the genome.
- The mechanism of hybridisation of the probe with target, and reaction kinetics, are well understood.
- A wide range of detection and amplification techniques is available.
- Sensitivity exceeds that of immunology by many orders of magnitude.

A number of disadvantages should be noted.

- Specialised facilities are needed.
- The required skill base is high.
- Nucleotide sequence information for the pathogen is needed.
- Troubleshooting is a constant feature of these methods, so that research needs to be combined with development.

**Table 1.** Evaluations<sup>a</sup> of indexing techniques based on immunological and nucleic acid methods.

Property	Immunological tests		Nucleic acid specific tests	
	Precipitin tests	Amplified	Probes	PCR amplified
Sensitivity	Low	Medium	High	Very high
Speed	High	Medium	Low	Low
Specificity	Medium	Medium	High	High
Cost	Low	Medium	High	High

<sup>a</sup> These are relative evaluations based on inherent characteristics of the tests, and assume that laboratory facilities and training levels are not limiting.

- Assays can be influenced by the molecular structure of the probe or target nucleic acid, and plant products such as enzymes and inhibitors.

To establish a nucleic acid based scheme, the minimum requirements are:

- to isolate and characterise the pathogen genome, or the component that is to be the target;
- to determine an appropriate strategy for indexing;
- to develop and produce specific probes or primers;
- to develop appropriate plant processing methods;
  - sampling and storage
  - extraction
  - selective concentration of the target
  - removal of contaminants;
- to develop detection methods
  - hybridisation strategy
  - amplification strategy
  - detection and scoring
  - further analysis of the target; and
- to interpret.

All of the above steps need to be optimised for a specific pathogen or group of pathogens, and a range of nucleic acid technologies is available for application to indexing. Selection can be made from the following range of methods.

- Hybridisation with randomly primed complementary DNA representing the full genomic RNA or DNA of the pathogen. This technique is most appropriate for viruses.
- Hybridisation with short cDNA probes representing either conserved or variable parts of the genome of the pathogen. This is appropriate for viruses and viroids, but its application still requires development.
- Cloning of pathogen nucleic acids to produce a cDNA or genomic library. Pathogen specific clones can be identified and sequenced. These can be subcloned into appropriate vectors and probes synthesised for specific applications by molecular hybridisation analysis. This method is very suitable for prokaryotic and eukaryotic organisms, and viruses.
- PCR requires knowledge of the sequence to be detected so as to allow the design of forward and reverse oligodeoxynucleotide primers that will bracket a target sequence (preferably 200–1000 nucleotides in length). Detection of an RNA target requires an initial reverse transcription step to cDNA prior to PCR and this may limit the sensitivity of the method. Although PCR is potentially extremely sensitive, workers frequently report interference by inhibitors in plant extracts. Analysis of the size and sequence of the product may be required to confirm its identity.

### Diseases of Unknown Etiology

Indexing by the above methods assumes that the pathogen to be identified has been isolated and characterised, and that relevant probes are available. Diseases

of unknown etiology require a different approach in which component analysis is again an important strategy. It is particularly useful for viruses where electron microscopy is unsuccessful (Randles 1993) as the size and structure of viral nucleic acid components is almost always different from that of normal host nucleic acids.

A range of techniques including gel electrophoretic fractionation, end-labelling, enzyme sensitivity, dye-binding, buoyant density, thermal denaturation profiles and electron microscopy is available to isolate and characterise disease associated nucleic acids. They can be typed to RNA or DNA, and their strandedness, linearity or circularity, and apparent size can then be described. A comparison with the known properties of virus genera and families (Francki et al. 1991) then allows the tentative identification of the virus to genus level. Identification of the putative replicative forms of RNA viruses, which can be readily identified as ds-RNAs, has been one of the most useful advances (Dodds 1993) as their stability allows quite rigorous purification. Circular viroids and satellites, and circular ssDNA and dsDNA are also readily separated by two-dimensional gel electrophoresis. Purification is an avenue to cloning and sequencing, and hence to a standard nucleic acid indexing procedure.

A general PCR based technique has been developed which may be applicable to diseases of unknown etiology with a possible phytoplasma etiology. The 16S ribosomal DNA (rDNA) sequences are amplified by PCR, using a 'universal' primer pair. For example, Lee et al. (1993) designed such primers for the specific amplification of 16S rDNA from a broad array of phytoplasmas from infected plant tissues, and then used restriction enzyme digestion of the products in an RFLP (restriction fragment length polymorphism) analysis for differentiating and classifying them. The phytoplasmas were therefore not cultured, and direct identification was possible from plant DNA extracts.

### Conclusions

New technologies based on immunological and molecular biological methods offer more rapid, more sensitive, more reliable and more efficient means of identifying parasites in plants. They can be used to set up specific tests for known pathogens, or used to identify a new pathogen where a disease of unknown etiology occurs. They do not assay pathogenicity, so additional experiments are needed if pathogenicity is to be tested. However, they may allow the amplification of the genome so that it may be inoculated in a pathogenicity test. A further justification for the use of new technologies is that they not only use current knowledge of pathogen composition for indexing, but they can also provide tools such as probes, which can be applied to more intricate studies of pathogen structure, host-pathogen interactions, and to epidemiology.

PCR has already made a major contribution to indexing for prokaryotic and eukaryotic pathogens. The strategy of amplifying specific genes and subsequently analysing them for sequence differences by either RFLP or direct sequencing can be expected to have a major impact on plant pathology. Likewise, probes for specific genes or sequences offer a new approach to identification and epidemiology.

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# Molecular Tools for Indexing in Sugarcane

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## Abstract

Indexing for diseases in sugarcane will be significantly enhanced by the application of specific molecular tests. In circumstances where the pathogen is present in low titre, symptomatology is unreliable, or the infection is latent, molecular tools provide the most sensitive and reliable method for detection. For some diseases of sugarcane, specific molecular tests have been developed to detect the pathogen, but the tests have not yet been applied to routine indexing. In other cases, the tests are still being developed, or the methodology exists but has not yet been applied to pathogens of sugarcane.

THE INTERNATIONAL EXCHANGE of sugarcane germplasm is essential for the success of breeding programs and research in many sugarcane growing countries. A secure and well-managed quarantine facility is essential to minimise the risk of introducing new diseases or new variants of diseases into sugarcane growing areas. Previously, most diseases of sugarcane were diagnosed on the basis of symptoms, and for some bacterial and viral pathogens, a serological based test was available to assist in identifying the causal agent. In recent years a number of new diseases of viral or unknown cause (for example, sugarcane bacilliform virus [SCBV], sugarcane mild mosaic virus [SCMMV] and yellow leaf syndrome) have been detected in sugarcane. At the same time, the advent of new molecular techniques provides an opportunity to improve quarantine security by providing sensitive specific tests for both new and well-known diseases. This is a short review of the molecular tests currently available, and of those being developed, for diagnosing different sugarcane diseases.

## Virus and Virus-Like Diseases

### Viruses

Sugarcane has a number of viral pathogens and, for most, either an antiserum based or a nucleic acid based test has been developed, as detailed in Table 1. For some viral diseases, diagnosis by symptomatology is unreliable. For example, the flecking symptoms associated with SCBV infection are variable or may be absent, and although Fiji disease virus causes characteristic galls, a long latent period may occur between infection and gall development. In other cases, viral titre is low, for example in SCBV-infected commercial canes. Molecular tests provide a means of diagnosing asymptomatic plants and plants containing low titres of viruses. Sensitive and specific molecular tests based on

the polymerase chain reaction (PCR) for DNA viruses such as sugarcane streak virus, or reverse transcriptase PCR for RNA viruses such as SCMMV and peanut clump virus can be developed once the nucleic acid sequence of the virus is known. This approach has been successfully used to develop PCR based tests for SCBV (Braithwaite, Egeskov and Smith 1995), and reverse transcriptase PCR based tests for Fiji disease virus and sugarcane mosaic virus (SCMV) (Smith and Van de Velde 1994). For most viruses listed in Table 1, DNA primers or probes are available, but a rapid, routine screening test suitable for indexing sugarcane in quarantine has yet to be developed.

Some viruses are known to exist as a number of strains and a practical indexing test must be able to detect all strains. Tests based on antisera are generally less sensitive than PCR based tests and can be strain-dependent. PCR primers targeted to conserved regions of the viral genome maximise detection of all isolates. Several strains of SCMV have been described and a range of polyclonal (Shukla et al. 1989) and monoclonal (Lin and Chen 1994) antisera are available to differentiate strains of the SCMV subgroup of potyviruses. The PCR primers developed to detect SCMV-infected cane are able to detect SCMV strains A, B, D and E.

### Unidentified viruses

Vegetatively propagated (clonal) grasses often contain unidentified, agronomically important, and sometimes symptomless viruses. Such viruses pose additional risks to the safe introduction of foreign germplasm. As described above, specific indexing tests for known viral pathogens of sugarcane are being developed. However, only general tests that are relatively insensitive, can be used to detect unidentified viruses. Sap inoculation of indicator plants and sap examination by electron microscopy are two traditional methods for detecting the presence of viruses, while methods to detect the presence of double-stranded RNA (dsRNA) and PCR tests using 'universal' primers are molecular tests also available to screen germplasm.

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Many plant viruses have a genome composed of dsRNA or produce dsRNA during replication. The number and size of dsRNA species can be diagnostic for a specific virus (Hansen and Wick 1993). PCR technology may also offer solutions for the detection of some unknown viruses. PCR primers complementary to conserved genomic sequences shared by all known members of a virus group can be used to identify related but undescribed members. Such primers are termed 'universal', 'group-specific' or 'degenerate' and have been developed for luteoviruses (Robertson, French and Gray 1991), potyviruses (Langeveld et al. 1991) and geminiviruses (Rybicki and Hughes 1990). Investigations to determine the causal agent of yellow leaf syndrome illustrate the potential of some of these techniques. Evidence obtained from sap examination under the transmission electron microscope and reverse transcriptase PCR amplification with luteovirus group-specific primers suggests that a luteovirus may be associated with the syndrome.

## Prokaryotic Diseases

### Bacteria

Bacterial pathogens of sugarcane are generally diagnosed on the basis of symptomatology and microscopy, and in many cases by a serological test such as enzyme-linked immunosorbent assay (ELISA) or immunofluorescence. However, several pathogens are currently the subject of molecular characterisation and consequently molecular tools are available that could be developed for indexing. Major bacterial pathogens that have a specific test available are listed in Table 2.

PCR based methods are being developed to detect the causal agent of ratoon stunting disease, *Clavibacter*

*xyli* ssp. *xyli*, in xylem exudate using short random primers (random amplified polymorphic DNA, RAPDs) (Petrasovits et al. 1994) or specific primers (Fegan et al. 1994). Sugarcane in Australia is routinely screened in testing laboratories for ratoon stunting disease as an industry service by a very sensitive evaporative binding enzyme-linked immunoassay technique (Croft et al. 1994). A diagnostic test for the bacteria is essential because the disease is associated with no specific external symptoms and internal symptoms may not occur in all clones.

*Xanthomonas albilineans*, the causal agent of leaf scald, may have a latent period and is difficult to isolate because of its fastidious nature. Enzyme-linked immunoassay and selective media are used to index the bacteria (Davis et al. 1994a). Variation between isolates collected throughout the world has been revealed by restriction fragment length polymorphisms (RFLPs) (Davis et al. 1994b).

*Xanthomonas campestris* pv. *vasculorum*, the causal agent of gumming disease, can be detected by DNA probes (Saumtally, Autrey and Daniels 1991) and serological techniques using a monoclonal antibody (Dookun, Jones and Autrey 1991).

### Mycoplasma-like organisms

White leaf and grassy shoot are caused by mycoplasma-like organisms (phytoplasmas). These organisms are diagnosed by symptomatology and examination of ultrathin sections by electron microscopy. There is a preliminary report of a monoclonal antibody for the white leaf pathogen (Clark and Sarindu 1991) and DNA probes (Klinkong and Seemüller 1992) (Table 2). A wide range of mycoplasma-like organisms can be detected by a PCR based test that uses a universal primer pair specific to the 16S ribosomal RNA

**Table 1.** Indexing tests for viral pathogens of sugarcane.

Pathogen	Disease	Molecular approach	Serological approach
Sugarcane mosaic virus (SCMV)	Mosaic	PCR primers (Smith and Van de Velde 1994)	Polyclonal (Shukla et al. 1989) Monoclonal (Lin and Chen 1994)
Sugarcane streak virus (SSV)	Streak	DNA probes (Hughes et al. 1992) Degenerate PCR primers (Rybicki and Hughes 1990)	Polyclonal and monoclonal (Dekker et al. 1988)
Fiji disease virus	Fiji disease	PCR primers (Smith and Van de Velde 1994)	Polyclonal (Wagih et al. 1995)
Sugarcane bacilliform virus (SCBV)		PCR primers (Braithwaite et al. 1995)	Polyclonal (Lockhart and Autrey 1988)
Sugarcane mild mosaic virus (SCMMV)	Mild mosaic		Polyclonal (Lockhart et al. 1992)
Peanut clump virus	Red leaf mottle		Polyclonal (Baudin and Chatenet 1988)

**Table 2.** Indexing tests for prokaryotic diseases of sugarcane.

Disease	Molecular approach	Serological approach
<b>Bacterial</b>		
Ratoon stunting	RAPDs (Petrasovits et al. 1994) PCR primers (Fegan et al. 1994)	EB-EIA (Croft et al. 1994)
Leaf scald	PCR primers (Lopes and Damann 1994)	EIA (Davis et al. 1994a)
Gumming	DNA probes (Saumtally et al. 1991)	Monoclonal antibody (Dookun et al. 1991)
<b>Mycoplasma-like organisms</b>		
White leaf	DNA probes (Klinkong and Seemüller 1992)	Monoclonal antibody (Clark and Sarindu 1991)
Grassy shoot	Universal primers (Lee et al. 1993)	

(rRNA) gene (Lee et al. 1993). A simple indexing test based on these primers could be developed for mycoplasma-like organisms of sugarcane.

### Fungal Diseases

A wide range of fungal diseases of sugarcane have been recorded, including downy mildew, smut, rust and red rot. Generally fungal diseases are diagnosed by symptomatology, followed by isolation and examination of the organism. Rapid diagnostic techniques such as PCR, immunofluorescence and ELISA are being developed to detect the fungal pathogens of many crops. However, the commercial development of these techniques is expensive and, since many fungi can be detected by conventional means, the cost cannot be justified in many cases (Hansen and Wick 1993). Molecular tools would have a useful role in detecting and identifying the root pathogens *Pythium* spp. and *Pachymetra chaunorhiza*. In general, soil pathogens are difficult to work with and members of the Oomycetes lack easily identifiable morphological characteristics. Two preliminary studies have been reported where PCR based techniques were used to distinguish isolates of root rot causing fungi of sugarcane. DNA amplification fingerprinting was used to distinguish isolates of *Pythium arrhenomanes* and *P. graminicola* (Bassam et al. 1994) and RAPDs were used to distinguish isolates of *Pachymetra chaunorhiza* (McGhie et al. 1994).

### Conclusions

For many pathogens, the technology to develop indexing tests is available but has not yet been applied to indexing quarantined sugarcane, and further work may be required before a diagnostic test is functional. The test must be simple and rapid for routine screening; the

optimal sampling strategy for pathogen detection must be determined; and the test must be sensitive enough to diagnose the pathogen in asymptomatic cane. The test could then be incorporated in a suite of indexing tests for sugarcane.

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# Indexing of Potato Tubers for the Presence of Ring Rot and Brown Rot

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## Abstract

Latent (symptomless) infections of potato tubers are ring rot, caused by *Clavibacter michiganensis* ssp. *sepedonicus*, and brown rot, caused by *Pseudomonas solanacearum* — diseases for which there is zero tolerance for purposes of seed certification. Tuber indexing requires adequate sampling methodology and laboratory testing procedures that are both sensitive and specific. To date, the detection of ring rot has depended on a combination of Gram staining, eggplant inoculation, and either immunofluorescence microscopy, enzyme-linked immunosorbent assay or latex agglutination tests. Molecular methods reliant on nucleic acid hybridisation and the polymerase chain reaction are being evaluated and show promise as means of increased specificity and sensitivity in testing for ring rot and brown rot in planting material.

TWO TUBER-BORNE BACTERIAL DISEASES of potato are of particular importance in local and international plant quarantine and the certification of planting material. Ring rot, caused by *Clavibacter michiganensis* ssp. *sepedonicus* (*Cms*) is confined mainly to temperate and cold regions of North America, Europe and the former USSR, whereas brown rot, caused by *Pseudomonas solanacearum* (*Ps*), occurs very widely where potato is grown at high elevation in the tropics and subtropics, as well as in Mediterranean and some cool temperate regions (Olsson 1976). For the purposes of seed certification there is zero tolerance for both pathogens. Both ring rot and brown rot are notorious for latent (symptomless) infections in seed potatoes. Direct losses due to both diseases may be substantial. For ring rot the cost is greatly compounded by expenditure on administrative, regulatory, phytosanitary and other measures, particularly in countries producing seed potatoes (Langerfeld 1989). Because of zero tolerance of infected tubers, tests that are specific and sufficiently sensitive to detect low levels of infection are required. Resistant or tolerant cultivars can act as symptomless carriers of both diseases. In some cases these cultivars harbour substantial populations of the pathogen (Ciampi and Sequeira 1980; Manzer and Kurowski 1992).

The first tuber indexing methods were based on distinctive staining properties and biological assays involving inoculation of susceptible indicator plants. Later serological methods were developed and most recently molecular methods reliant on hybridisation of nucleic acids and the polymerase chain reaction (PCR). This paper assesses the efficacy and present

status of these methods in disease diagnosis and tuber indexing.

## Staining Properties

Potato tubers at an advanced stage of infection with ring rot or brown rot may show similar symptoms in the ring of vascular tissue exposed when tubers are cut transversely, and it is sometimes difficult to distinguish the two diseases (Harrison 1961). Ooze from the eyes is usual in bacterial wilt and absent in ring rot. In bacterial wilt there is usually brown discoloured vascular tissue and a milky exudate of bacterial ooze from the vascular ring that is increased by squeezing the tuber between thumb and forefinger. With ring rot tuber sections also show browning of the vascular system that exudes bacterial ooze when squeezed. By contrast, with bacterial wilt, most or all of the tuber vascular ring is rotted and turns either gray, yellowish, tan or reddish brown, and the bacterial ooze is pale, creamy-yellow, and of a cheesy consistency. However, with bacterial wilt as with ring rot at an advanced stage there may be cavitation in the vascular ring. In these circumstances staining reactions provide valuable confirmatory evidence. In stained smears *Cms* is Gram-positive consisting of coccoid and occasionally coryneform or elbow-shaped cells, whereas *Ps* is a Gram-negative rod containing poly- $\beta$ -hydroxybutyrate inclusions. These inclusions appear as refractile bodies under phase microscopy. Smears are stained with Nile blue A followed by examination under oil immersion with epifluorescence at 450 nm for granules that fluoresce bright orange (Ostle and Holt 1982). Nile blue A staining is more reliable than Sudan Black B staining (Lelliott and Stead 1987).

Staining methods are useful aids in disease diagnosis but alone are insufficient for indexing potato tubers. The methods lack specificity. A variety of

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Gram-positive bacteria including coryneforms is known to occur as endophytes in healthy tubers and as secondary invaders in diseased or decaying tissue (Shepard and Claflin 1975; De Boer 1991).

### Biological Assays

Inoculation into eggplant (*Solanum melongena* L. cv. Black Beauty) is considered to be the most suitable biological assay for *Cms* (Lelliott and Sellar 1976; Olsson 1976; Zeller and Xie 1985). Young plants about 8–10 cm high, approximately at the stage of having the third true leaf half expanded, are sensitive to very low populations of *Cms*. Sectoral wilting occurs, which may be preceded by large, greasy lesions in the interveinal areas (Lelliott and Stead 1987). Symptoms develop more rapidly in plants grown under controlled conditions in a growth chamber than in plants grown in a greenhouse (Bishop and Slack 1987). Confirmation of identity can then be made from symptomatic tissue of wilted eggplants using the Gram stain and serological tests. The presence of *Cms* in latently infected potato can be established by extracting bacteria from bulked core samples from the heel ends of potato tubers followed by eggplant inoculation (Lelliott and Sellar 1976). In potato tissue the threshold concentration of bacteria necessary to initiate infection in eggplant is about  $10^4$  cfu ml<sup>-1</sup> (Zeller and Xie 1985). Under appropriate conditions the sensitivity may be much greater, with ED<sub>50</sub> < 10 cfu (Lelliott and Sellar 1976).

In indexing for bacterial wilt of potato caused by *Ps* externally and internally symptomless tubers are planted in pots and maintained at temperatures optimal for disease expression. Alternatively symptomless tubers are incubated at 28–30°C in sealed desiccators containing free water and examined daily for release of bacterial ooze from the 'eyes' (Graham, Jones and Lloyd 1979), or stored at 18–20°C for three weeks (Nyangeri, Gathuru and Mukunya 1984).

### Immunological Methods

Immunofluorescence microscopy, enzyme-linked immunosorbent assay (ELISA), latex agglutination and Ouchterlony double diffusion tests have all been used for detecting latent tuber infection (Corbière, Hingand and Jouan 1987; De Boer and McNaughton 1986; De Boer et al. 1992a, 1992b, 1994; Miller 1984a; Slack, Kelman and Perry 1978; Zielke and Kalinina 1988). Of these the last two methods are not sensitive to a low number of cells (Bishop and Slack 1987).

The detection limit of ELISA for *Cms* has been reported to be in the range of  $1 \times 10^5$  to  $5 \times 10^6$  cfu ml<sup>-1</sup> (Corbière et al. 1987; Zielke and Kalinina 1988). The disease can be detected in seed potato lots by serological tests with greater accuracy than by visual field

inspections. The practical limit of immunofluorescence microscopy is generally found to be in the range of  $10^4$  to  $10^3$  cfu ml<sup>-1</sup> (De Boer 1991). The disadvantage of serological procedures is that they lack absolute specificity, since cross-reacting bacteria have been isolated from potato tissue. The occurrence of low numbers of fluorescing coryneform bacteria located by immunofluorescence microscopy in heel-end extracts of healthy potatoes was shown to be a normal phenomenon (Miller 1984b). Other disadvantages are the possibility of operator error and the cost of reagents used. In cases of doubt with the immunofluorescence test potato extracts should be submitted to the eggplant inoculation test.

Both polyclonal and monoclonal antibodies have been developed to test for *Ps* in brown rot of potato. When used in an indirect ELISA, the polyclonal antibodies detected as few as  $1 \times 10^4$  cfu ml<sup>-1</sup> in infected plant or soil samples, but there were cross-reactions with some related saprophytic bacteria. Improved specificity was obtained with monoclonal antibodies but they were of reduced sensitivity, detecting down to only  $1 \times 10^6$  cfu ml<sup>-1</sup> (Robinson-Smith et al. 1995).

### Molecular Methods

Conserved nucleotide sequences have been sought in plasmid and chromosomal DNA that could serve as species-specific probes for direct application in nucleic acid hybridisation (Johansen, Rasmussen and Heide 1989) and to generate primer sequences for use in PCR. These methods hold promise of greater rapidity and specificity.

In the case of ring rot, sequences have been obtained from a subcloned DNA fragment of a highly conserved plasmid of *Cms* and a pair of oligonucleotides constructed for use as PCR primers. PCR carried out using this primer pair and untreated cells of all strains of *Cms* resulted in the amplification of a DNA fragment of about 670 base pairs. No amplification was observed when bacteria belonging to other species were submitted to PCR under the same conditions. The detection limit of the assay was  $4 \times 10^3$  cfu ml<sup>-1</sup> (Firrao and Locci 1994). Similar results were obtained by Schneider, Zhao and Orser (1993) using different primers that generated a 258 bp fragment in all strains of *Cms* except one strain that was plasmidless.

The ribosomal RNA genes provide nucleotide sequences that are either fully conserved, semiconserved or variable between species. Mirza et al. (1993) amplified by PCR a 400 bp fragment of a 16S rRNA gene that covered two variable regions important for oligonucleotide probe development. A specific 24 mer oligonucleotide probe targeting the V6 region of 16S rRNA was designed and evaluated using dot blot hybridisation. Under stringent conditions (60°C) the probe hybridised only with *Cms* and no related bacteria. At low stringency (40°C) all subspecies of

*Clavibacter michiganensis* hybridised, suggesting that under these conditions the probe may be used as a species-specific probe for *C. michiganensis*. A DNA hybridisation probe was less sensitive than ELISA for detecting *Cms* in plant tissue but more reliable because of the absence of false positive reactions (Drennan et al. 1993).

Molecular methods have also been successfully applied to the diagnosis and detection of brown rot in potato. PCR assays directed at transfer RNA consensus sequences or at DNA sequences specific to *Ps* have been used successfully to screen for the bacterium in potato crops (Seal, Jackson and Daniels 1992a, 1992b). Equal success has been obtained using primers based on the polygalacturonase gene, which is essential for pathogenicity (Gillings, Fahy and Davies 1993). Species-specific primers have also been obtained from analysis of partial (Seal et al. 1993) and total sequencing of the 16S rRNA gene in *Ps* (Li et al. 1993; Taghavi, Fegan and Hayward 1994). Two sets of primers were designed to detect *Ps* belonging to the restriction fragment length polymorphism (RFLP) divisions I and II of Cook, Barlow and Sequeira (1989). Following optimisation of the conditions of PCR all strains of *Ps* were distributed within the two divisions as expected. The limit of sensitivity of the division-specific primers was about  $10^3$  cfu ml<sup>-1</sup>. A cloned random amplified polymorphic DNA (RAPD) band has been sequenced and used as the basis for primer development. The RAPD primer pair AU759/760 generated a 281 bp DNA fragment that appears to be specific for *Ps* and a few related species. This primer gave visible amplification products with between 1–20 cells in fresh sap from infected tomato plants. The PCR method was a quicker and more reliable method of detecting *Ps* than either a cultural method (spiral plating) or the use of ELISA. Although the primer pair based on 16S rRNA sequences was less sensitive than AU759/760 it appears to be more specific, because the latter primers sometimes gave amplification products even from healthy samples.

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# The Victorian Certified Seed Potato Scheme

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## Abstract

The Victorian Certified Seed Potato Scheme has been in operation since 1937. Today, tissue culture is used to eliminate pathogens, rapidly multiply stocks, and maintain pathogen tested stocks. The scheme has been successful in helping to control the major tuber-borne bacterial, fungal and virus diseases of potato known to occur in Australia.

THE PATHOGEN TESTED APPROACH to disease control can be applied to all vegetatively propagated plant species. This approach is the only way to control virus diseases and is an important management strategy for many fungal and bacterial diseases. The pathogen tested approach is regarded as an important form of administrative disease control (Ebbels 1979) and the first pathogen tested schemes began in the early part of the century. Certification schemes for potatoes were established before 1914 in Germany (Appel 1934), in the United States of America and Canada between 1913 and 1915 (Shepherd and Claflin 1975), and in the United Kingdom in 1918 (Ebbels 1979). These schemes were initially established to ensure varietal purity. In 1932 the UK scheme began to consider the health status of potatoes and the first virus tested stocks were introduced in 1945 (McIntosh 1951).

The success of the seed certification schemes for potatoes soon prompted the application of this approach for the quality control of plant material to other suitable crops. By 1960 schemes were in place all around the world for fruit (strawberry runners, raspberry canes, blackcurrant bushes, hops, avocados, stone and pome fruit) and ornamental crops (narcissus, iris, carnations and chrysanthemums) (Ebbels 1979). This expansion to other crops has continued and today most vegetatively propagated crops have some form of pathogen tested scheme.

A complete review of pathogen tested schemes for all crops is beyond the scope of this paper. Therefore the Victorian Certified Seed Potato Scheme will be used as a model for the development of a pathogen tested scheme. A brief history of the scheme will be given along with some technical details.

## History

The Department of Agriculture Victoria established a Certified Seed Potato Scheme in 1937, which initially

involved certification of crops based on visual inspection. This scheme succeeded in significantly reducing the incidence of severe mosaic diseases such as potato viruses A (PVA), Y (PVY) and leafroll (PLRV). However, certification by visual inspection alone was not controlling the important tuber-borne bacterial and fungal pathogens or the mild and latent virus diseases, potato viruses X (PVX) and S (PVS).

In 1967 a new approach was adopted. Clones of selected cultivars were tested for known pathogens and those clones in which no pathogens were detected formed the mother stocks for the first pathogen tested seed scheme. Mother stocks were maintained in an insect proof glasshouse and planting material was multiplied by stem cuttings. These cuttings were supplied to foundation seed growers, who multiplied them in the field for four generations. This foundation seed was then sold to certified seed growers, who grew them for a further two generations. These crops were inspected twice during the growing season. Tubers were also inspected and if they were up to standard they were certified as seed.

In 1980 the scheme was modified. Tissue culture was introduced as a means of eliminating pathogens, rapidly multiplying stocks for growers and maintaining the pathogen tested lines in long term storage. Foundation growers were supplied with minitubers instead of stem cuttings. In 1986 the production of minitubers was increased tenfold and field multiplication was reduced by one generation to reduce field exposure to disease.

## Virus Elimination

Viruses are eliminated from potatoes using a combination of heat therapy and meristem culture. Prior to this treatment potatoes are tested for the presence of potato spindle tuber viroid (PSTV), which is not known to occur in Australia. Any plants that are found to be infected with PSTV are discarded. Viroids are warm climate pathogens and replicate at higher rates under high temperature conditions (Singh 1983). The use of heat therapy and meristem culture results in low proportions of uninfected plants (Stace-Smith and Mello

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1978). However, more success has been had with a combination of cold treatment and meristem culture (Paduch-Cichal and Krycznski 1987).

Potato plants are placed in a heat treatment room at 38°C for approximately three months. A second PSTV test is done at this stage to ensure that no PSTV has escaped detection. Meristems from these plants are removed and grown in-vitro in liquid medium. In-vitro plantlets are grown on solid media and an initial test for virus is done. If no virus is detected then the plantlets are grown in soil in a glasshouse. A second round of virus testing is done when the plants are growing vigorously.

### Virus Indexing

All plants are closely monitored visually for any unusual symptoms. Plants are tested for PVA, PVM, PVS, PVY, PVX, PLRV and tomato spotted wilt virus using the enzyme-linked immunosorbent assay (ELISA). However, there are many other viruses known to infect potatoes for which ELISA is unavailable. Therefore the plants are also tested by mechanical inoculation to a range of herbaceous indicator species.

New tests are introduced when appropriate, for example a polymerase chain reaction test for PSTV is being developed. Plants are currently tested for the presence of PSTV using a biotin labelled cDNA probe.

### Effectiveness

There have been two major benefits of the scheme. The first is the ease with which a small amount of healthy

material can be rapidly multiplied. It is quite easy to produce over one tonne of tubers from one tissue culture plantlet within twelve months.

The second has been the reduction in losses caused by disease. All the major tuber-borne diseases of potato known to occur in Australia have been controlled to some degree by this scheme. PVA, PVM, PVX and PVY have ceased to be detectable in Victorian seed crops. PLRV and PVS, once widespread, have been reduced to sporadic outbreaks.

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# The Safe Movement of *Musa* Germplasm

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## Abstract

The INIBAP Transit Centre in the Laboratory of Tropical Crop Husbandry at the Katholieke Universiteit Leuven, Belgium, holds the world's largest in-vitro collection of *Musa* germplasm under slow growth conditions. One of INIBAP's key functions is to provide superior land races and improved germplasm to Natural Agricultural Research Systems (NARS) for evaluation. Accessions are distributed either as in-vitro rooted plantlets or as proliferating tissue. This eliminates many disease risks, but viruses pose a problem as they can be carried in tissue culture without symptom expression. Accessions are tested for viruses at INIBAP Virus Indexing Centres, which are located at CIRAD-FLHOR (Montpellier, France), the Queensland Department of Primary Industries (Brisbane, Australia) and TBRI (Pingtung, Taiwan) before distribution. The main concerns are banana bunchy top virus, banana streak virus, banana bract mosaic virus and cucumber mosaic virus, but uncharacterised viruses may also be present. INIBAP will follow the new FAO and IPGRI technical guidelines for the safe movement of *Musa* germplasm, which are very similar to protocols currently employed at INIBAP Virus Indexing Centres.

GERMPLASM EXCHANGE has always been one of the most important functions of the International Network for the Improvement of Banana and Plantain (INIBAP). The focus of this activity is INIBAP's Transit Centre in the Laboratory of Tropical Crop Improvement at the Katholieke Universiteit Leuven, Belgium. Here, *Musa* accessions representing the diversity of the genus are stored under slow growth conditions ( $15 \pm 1^\circ\text{C}$ , 2000 lux) as part of INIBAP's *Musa* germplasm conservation network.

Each accession is represented by 20 tissue culture tubes, which are all regularly checked for contamination. When the number of tubes is reduced to 12 by attrition, the accession is placed under optimum growth conditions ( $28 \pm 2^\circ\text{C}$ , 5000 lux) and reactivated. After multiplication, 20 tubes are reestablished in the collection.

Under slow growth conditions, accessions are subcultured only once a year on average (De Smet and Van den houwe 1991). However, while some can be stored for 615 days, others need to be subcultured every 60 days (Van den houwe et al. 1995). Differences in storage potential relates to the genomic composition of the accession. Cultivars in the East African Highland group (AAA 'Mutika/Lujugira') and AAB banana types (other than plantain cultivars) can be stored much longer than all other genotypes. In general, wild *Musa* species need to be subcultured the most frequently, with *M. balbisiana* having the shortest storage time (Van den houwe et al. 1995).

The Transit Centre started its activities in 1985 with a core collection of 17 accessions. Today, over 1000 accessions of wild species, land races, hybrids and genetic variants (developed through somaclonal and mutation breeding) are held at the Transit Centre, making it the largest in-vitro *Musa* genebank in the world.

## Germplasm Distribution

Since 1985 the number of requests for germplasm from NARS, universities, regional institutes, international institutes and botanical gardens has been steadily increasing (Figure 1). In recent years the Transit Centre has also been involved with the supply of germplasm for multilocal trials in association with INIBAP's International *Musa* Testing Program, for multilocal taxonomic studies related to INIBAP's *Musa* Germplasm Information System and for the duplication of part of the collection in Taiwan for safety reasons.

The general distribution of germplasm falls under INIBAP's *Musa* Germplasm Exchange System. Requests for material are either sent to INIBAP's Regional Coordinators in the Latin America/Caribbean and Asia/Pacific regions or to INIBAP's Germplasm Officer at headquarters in Montpellier, France. Requests to Regional Coordinators are forwarded to the Germplasm Officer, who, after determining if the accessions are available and virus tested, passes the order onto the officer in charge of the Transit Centre. Copies of this order are faxed or mailed to individuals or organisations requesting the germplasm for their information.

Germplasm can be dispatched as either proliferating tissue cultures or rooted plantlets. Normally five cultures or tubes of each are sent. The time required to fill

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an order depends on the type of culture required. Because accessions are stored under slow growth conditions and need to be reactivated for multiplication, it usually takes two months for proliferating cultures and three months for rooted plantlets. Rooted plantlets are distributed in Cultu saks® and proliferating cultures, which can be used by the customer for further multiplication in tissue culture laboratories, in tubes. Instructions on how to acclimatise plantlets are enclosed with Cultu saks®. All consignments contain a Belgium phytosanitary certificate and are sent by courier. In the future, details of virus indexing tests undertaken on accessions and other pertinent quarantine information will be provided for each accession exported.

There are plans to provide customers with an order form on which the germplasm transfer conditions are listed. This move is in response to the United Nations Convention on Biological Diversity, which recognises rights of the country of origin of genetic resources.

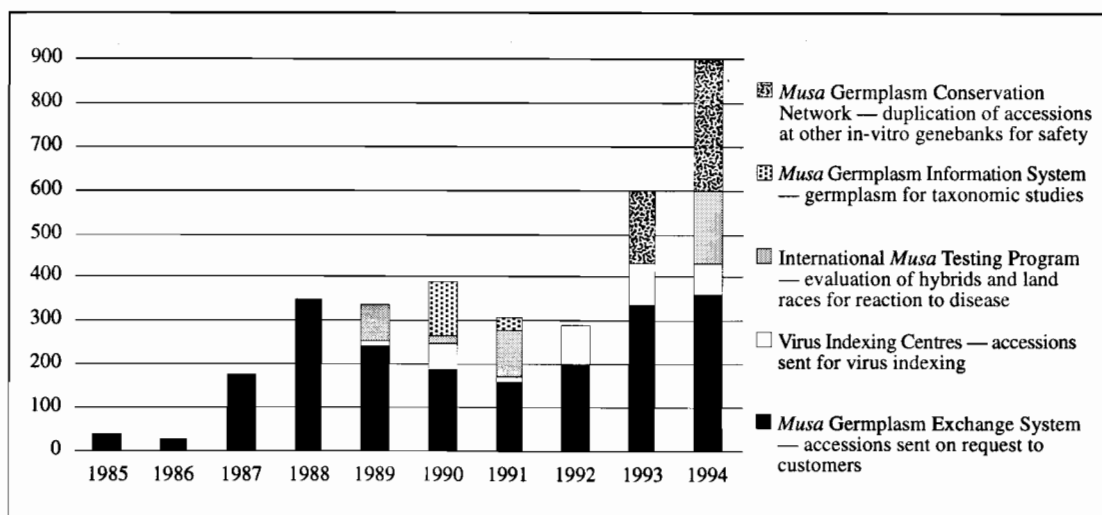
More information on *Musa* germplasm distribution from the Transit Centre is provided by Van den houwe and Jones (1994).

### Constraints on the Movement of *Musa*

The safe movement of *Musa* germplasm has been reviewed by Stover (1977), who highlighted the dangers posed by the transfer of material as suckers and corm pieces and emphasised the advantages of tissue cultures. Although tissue cultures eliminate the risk of moving pests, fungal and bacterial diseases and nematodes because, if present, they would contaminate the culture medium, the possibility of transferring virus diseases still exists.

In 1988 the Food and Agricultural Organisation of the United Nations (FAO), the International Board for Plant Genetic Resources (IBPGR) and INIBAP organised a meeting of *Musa* virologists and quarantine experts at Los Baños in the Philippines to discuss issues associated with the international movement of *Musa* germplasm and recommend protocols for ensuring safe transfer. These protocols were reported in guidelines edited by Frison and Putter (1989) and became the international standards. INIBAP has used these guidelines as a basis for movement, but has built on the recommendations as new information on *Musa* viruses has become available from partners and collaborators. At the time of writing, the FAO, the International Plant Genetic Resources Institute (IPGRI) and INIBAP were organising another meeting of *Musa* virologists to update protocols, which will be published in a second edition of the guidelines (see 'Post-script').

Viruses that can be carried in tissue culture have been described previously (Jones 1994) and will not be reported in detail here. However, the four main pathogens of concern are banana bunchy top virus, cucumber mosaic virus, banana bract mosaic virus and banana streak virus. In 1988, emphasis was placed on protocols to detect banana bunchy top virus as this was perceived as the major threat at this time. In 1990 a workshop in Montpellier, France, was organised by INIBAP and the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) with FAO and IBPGR involvement to compare banana bunchy top virus indexing methodologies and further refine the protocols for the guidelines (Iskra-Caruana 1994). One of the recommendations was to hold plants in quarantine



**Figure 1.** Number of accessions distributed from the INIBAP Transit Centre, 1985 to 1994 (Van den houwe 1995).



at 32°C under high light intensity, which are conditions for the optimum expression of symptoms of banana bunchy top disease.

Banana bunchy top disease was originally deemed to be of the greatest importance because it is the most serious virus disease of *Musa* and it was not present in the Americas where the major export plantations are located. However, since 1990, banana streak virus has become more of an immediate problem. Previously thought to be confined to a few African countries, it is now known to occur worldwide, though its significance seems to depend on the location. In some regions where mealy bug vectors are active, banana streak virus is spreading and can cause significant losses, but in other areas, symptoms are seen in only one or two cultivars and it seems to be spread mainly with propagating material. Banana streak virus has infiltrated at least three major *Musa* breeding programs and, being seed borne, has been found in new hybrids.

Banana streak virus has been detected in 45 accessions held at the Transit Centre. In contrast, banana bunchy top disease has not been found in any accessions, probably because symptoms of this disease are obvious and diseased plants would be avoided by most scientists in the field during selection for conservation and experimentation. Also, although the existence of latent and mild infections of banana bunchy top virus has been advocated (Su, Wu and Tsao 1993), there is still no unequivocal proof that they exist in the field. It is possible that false positive reactions in antibody tests, which may be associated with the absence of an antioxidant in the extraction buffer, may account for these claims. Latent infections have been reported in plantlets in tissue culture (Drew et al. 1989), but this is not a 'natural' environment and infected plants develop symptoms when grown in vivo. On the other hand, a characteristic of banana streak virus infection is the periodicity of symptoms expression. Plants may not show symptoms on all leaves and, for several months at a time, emerging leaves may be symptomless or show only slight symptoms (Jones and Lockhart 1993). Therefore, it is more likely for plants affected by the banana streak virus to be selected for shoot-tip culture, propagation and distribution than plants with banana bunchy top disease.

In Honduras, symptoms become apparent only during the cooler months and at other times infected plants can be symptomless. For this reason, INIBAP no longer advocates holding banana plants at 32°C in quarantine; it believes that they should be kept at 25–28°C, which is thought to be more appropriate for the development of banana streak virus symptoms. This temperature range is optimal for *Musa* growth and is also believed more suited to the expression of cucumber mosaic virus and banana bract mosaic virus symptoms.

## INIBAP's Virus Indexing Centres

INIBAP has developed a virus indexing system based on recommendations made at the FAO, IBPGR and INIBAP meeting on the safe movement of *Musa* germplasm held in Los Baños in 1988 (Frison and Putter 1989). Three Virus Indexing Centres have been established with key partners with expertise in *Musa* viruses. These centres are contracted to undertake indexing of Transit Centre accessions on INIBAP's behalf and are funded by grants from the Virus Indexing Centre host country. The three centres are located with the Queensland Department of Primary Industries, Brisbane, Australia (officer in charge: Dr John Thomas), CIRAD-FLHOR, Montpellier, France (officer in charge: Dr Marie-Line Caruana) and TBRI, Chiuju, Taiwan (officer in charge: Dr Sin Wan Lee). Five plantlets of each accession to be indexed are sent to a Virus Indexing Centre. All are grown in a quarantine glasshouse, but only four are indexed; the fifth is held in reserve in case of the death of a test plant. The indexing procedures followed at Virus Indexing Centres have been outlined by Jones and Iskra-Caruana (1994).

It is believed that the four plants are a representative sample of the material held in the Transit Centre as they are derived from cultures in a number of tubes, which in turn were produced from one introduced shoot tip. If the original germplasm in the Transit Centre were infected, then it is believed that at least one, and most likely all, of the test plants would carry the virus. Although it could be argued that, by chance, all of the plantlets sent to an indexing centre could be derived from primordial cells that have avoided contamination by a virus that infects other cultures of the same accessions, INIBAP believes that this is highly unlikely in practice. In 95% of cases, all test plants of an infected accession at Virus Indexing Centres have been found with virus. In the other 5%, three or two of the plants were infected.

If an accession originates in a country where banana bunchy top disease is known to occur, then plantlets of that accession are sent to two Virus Indexing Centres. This was one of the original recommendations made at the 1988 meeting in Los Baños and was advocated because of the importance of banana bunchy top disease and the possibility of latent strains of the banana bunchy top virus. Plantlets of an accession from countries where the banana bunchy top virus does not occur are sent to one Virus Indexing Centre. Plants are indexed twice during a 9–12 month period in quarantine for banana bunchy top virus and cucumber mosaic virus for which antisera are commercially available. This usually occurs after three months growth and before release. Accessions from the Americas are not indexed for banana bunchy top virus as the virus is not present on the continents. During quarantine, leaf sap is extracted, partially purified and examined under

the electron microscope for virus particles. Immunosorbent electron microscopy is also undertaken if banana streak virus is suspected as antisera to several strains have been supplied to Virus Indexing Centres by Dr B.E. Lockhart.

During the quarantine period, the plants are also inspected regularly for symptoms of virus infection. When an accession has been cleared by a Virus Indexing Centre, the officer in charge notifies the Scientific Research Coordinator at INIBAP headquarters in Montpellier, who enters the information in a computer file and changes the health status classification (see 'Health Status of Accessions').

The list of accessions that are available for distribution is updated twice a year and is distributed to INIBAP's partners and collaborators.

### Health Status of Accessions

Most accessions sent to the Transit Centre for inclusion in the collection are unindexed. However, there have been notable exceptions, such as the germplasm collected in Papua New Guinea by the International Board of Plant Genetic Resources, the Queensland Department of Primary Industries and the Papua New Guinea Department of Agriculture and Livestock in 1988-89, which was tested for cucumber mosaic virus while in quarantine in Brisbane, Queensland.

Accessions in the Transit Centre are placed in four categories depending on their health status.

#### 1. Available (virus indexed)

There are accessions held at the Transit Centre that have been virus tested to INIBAP standards and are available for distribution. In March 1995 there were 372 accessions in this category.

#### 2. Unavailable (to be indexed at one Virus Indexing Centre)

These accessions are either from countries where banana bunchy top disease is not known to occur, but still need to be indexed for other *Musa* viruses, or they are from countries where banana bunchy top disease is known to occur, but have been indexed already at one Virus Indexing Centre for banana bunchy top virus and other *Musa* viruses. There were 537 accessions in this category in March 1995.

#### 3. Unavailable (to be indexed at two Virus Indexing Centres)

These accessions are from countries with banana bunchy top disease and need to be virus indexed at two Virus Indexing Centres as a double check. There were 82 accessions in this category in March 1995.

#### 4. Virus infected

These accessions have been found to be infected with virus at Virus Indexing Centres. Because they are

important accessions, they are being held at the Transit Centre awaiting development of therapeutic protocols to eliminate the virus. Sixty-two accessions were in this category in March 1995.

### Postscript

A second meeting on *Musa* virologists and quarantine experts took place in Rome on 21-23 June 1995 under the auspices of the FAO, IPGRI and INIBAP to update the technical guidelines for the safe movement of *Musa* germplasm first developed in 1988. New protocols were proposed that generally follow those currently employed at INIBAP Virus Indexing Centres and described in this article. However, recommendations that differ from present procedures were made and these will be incorporated in INIBAP's protocols. The recommendations are as follows.

- It was considered that the five plantlets dispatched from the Transit Centre to Virus Indexing Centres for virus indexing should be selected from seven (not twenty) cultures derived from the original shoot-tip as this would lessen the risk of test plants being unrepresentative of the health status of the original accession. The modification will necessitate the multiplication of the remaining two cultures of the accession to obtain twenty cultures for holding under slow growth conditions at the Transit Centre.
- It was thought that the indexing period at Virus Indexing Centres should be reduced from 9-12 months to 6 months and that indexing should take place after 3 and 6 months growth because of increased confidence in the reliability of tests to detect virus pathogens, even as latent infection. It was also agreed that accessions from countries with banana bunchy top virus needed to be virus indexed at one Virus Indexing Centre only and not two as previously recommended. New antisera developed by Drs John Thomas and Marie-Line Caruana for banana bract mosaic virus will be routinely used at Virus Indexing Centres in the near future, together with a wide spectrum antiserum for detecting banana streak virus by immunosorbent electron microscopy developed by Dr Ben Lockhart in an INIBAP sponsored project. Partially purified leaf sap will still need to be examined under an electron microscope to detect particles of uncharacterised viruses.

Germplasm would be tested for all viruses according to the protocols specified in the new guidelines. However, in some instances, tests may be waived if there is strong, reliable evidence that particular viruses are not present in the country of origin of the germplasm.

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# Indexing and Establishing Disease-Free Germplasm of Pome and Stone Fruit Crops

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## Abstract

Quarantines for pome and stone fruit germplasm are long, costly and complex. The indexing of germplasm of both fruit groups relies primarily on graft transmission tests to woody indicator hosts. These tests are performed in greenhouses and in orchards, the latter requiring 3–5 years to complete. Both fruit groups have numerous diseases for which the causal agent is unknown. However, the two fruit groups are different in several ways that affect the quarantine process. Several of the submicroscopic pathogens that infect *Prunus* spp. have natural mechanisms of field spread, such as aphid transmission and by pollen; only phytoplasmas in pome fruits are spread by mechanisms other than graft transmission. The incidence of virus-like diseases has been less than 10% in *Prunus* spp., but more than 60% in *Malus* and *Pyrus* spp. This creates the need for a thermotherapy program for imported pome fruit germplasm. The treatment and reindexing can add 1–3 years to the quarantine period while increasing the maintenance and indexing workloads. The molecular techniques under investigation to expedite indexing are digoxigenin-labelled cRNA probes and nested polymerase chain reaction DNA amplification for viroid and phytoplasma detection, respectively.

THE AGRICULTURAL RESEARCH SERVICE (ARS) and the Animal and Plant Health Inspection Service (APHIS), two sister agencies within the US Department of Agriculture, have worked together since the early 1980s to create a consolidated centre for the quarantine and indexing of plants whose importation for propagative purposes is prohibited (US Department of Agriculture 1989). The centre is known as the National Plant Germplasm Quarantine Center and is located on the east campus of the Beltsville Agricultural Research Center (Building 580). Funding for the ongoing construction has been provided by APHIS. The quarantine and testing is conducted by ARS personnel in the National Germplasm Resources Laboratory (NGRL). The incentive for this cooperative effort was partly due to the increased demands placed on quarantine programs by the growing interest in the United States, and globally, in plant germplasm collection and preservation. The advantages and disadvantages of consolidated quarantine programs for numerous crops may be a topic for further consideration.

The quarantine unit of NGRL is responsible for the isolation and indexing of over 50 plant species including pome and stone fruits, potatoes, sweet potatoes, sugarcane, small fruits, rice and grasses (Waterworth 1993). The pome fruit and stone fruit are the most time consuming, difficult and expensive to index. While the general management and approach to the testing are similar for each of the two fruit groups, some problems

are unique to each. This paper considers these similarities and differences. The issues raised may serve as guidelines for developing improved quarantine methods for other crops, such as sugarcane.

## Germplasm Acquisition and Maintenance

The origins (donor countries) of germplasm received since 1990 are shown in Tables 1 and 2. From these data it is obvious that the quantity and source of the imported germplasm varies annually. The variations often reflect global and national political events and are difficult to predict. Germplasm is often exported from countries where records of endemic diseases are minimal or difficult to obtain because of linguistic problems. The former is often the problem when wild species are collected in developing countries.

Fruit tree germplasm generally is exchanged as dormant bud sticks. The quarantine period is typically 3–5 years, but an accession may remain in the program for 5–7 years to ensure that recipients and clonal repositories have established trees from the released and distributed propagation material.

Usually it takes one growing season to establish trees large enough to provide samples for indexing. Pome fruit trees are established directly in the orchard, while *Prunus* spp. are maintained in screenhouses with 30 mesh screen. The difference in management is due to the means of natural spread of pathogens in the different genera. Several pathogens in *Prunus* have mechanisms of field transmission, including plum pox virus by aphids and ilarviruses by pollen. Since field indexing can serve as a point of release for *Prunus* pathogens, these tests are not started until the greenhouse and laboratory tests are completed and the results are

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**Table 1.** Annual acquisition of *Prunus* spp. germplasm by origin.

Donor country	Year of acquisition						Total by country
	1990	1991	1992	1993	1994	1995	
Armenia	0	0	0	2	0	0	2
Bulgaria	0	0	1	0	0	0	1
China	0	13	16	4	11	3	47
Czech Republic	0	0	0	0	15	4	19
Ecuador	0	1	0	0	0	0	1
France	0	0	0	4	0	0	4
Germany	0	0	0	0	0	7	7
Greece	6	0	0	0	0	0	6
Hungary	0	0	1	0	14	10	25
Italy	2	0	0	9	0	19	30
Japan	0	1	0	0	0	0	1
Kazakhstan	0	0	4	2	0	0	6
Mexico	0	0	0	6	0	0	6
Nepal	1	0	0	0	0	0	1
Netherlands	0	0	3	0	0	0	3
New Zealand	5	0	0	0	0	0	5
Pakistan	0	0	1	0	0	0	1
Romania	0	12	12	0	6	1	31
Russian Federation	82	4	0	0	10	0	96
Spain	2	0	6	0	2	0	10
Sweden	0	2	1	0	0	0	3
Turkey	1	0	0	0	0	0	1
Turkmenistan	0	9	0	11	14	0	34
Ukraine	0	0	0	0	21	0	21
United Kingdom	0	0	0	0	0	1	1
<b>Total by year</b>	<b>99</b>	<b>40</b>	<b>46</b>	<b>39</b>	<b>93</b>	<b>45</b>	<b>362</b>

**Table 2.** Annual acquisition of pome fruit (*Malus*, *Pyrus* and *Cydonia* spp.) germplasm.

Donor country	Year of acquisition						Total by country
	1990	1991	1992	1993	1994	1995	
Brazil	11	0	0	0	0	0	11
Bulgaria	0	0	0	4	0	1	5
Canada	0	0	0	0	1	0	1
China	6	8	0	0	6	0	20
Czech Republic	0	0	0	0	1	0	1
Italy	0	0	0	10	0	0	10
Japan	5	0	10	5	2	0	22
Kazakhstan	0	0	0	11	0	0	11
Russian Federation	0	12	2	0	0	8	22
Sweden	0	1	0	0	0	0	1
Ukraine	0	0	0	8	0	0	8
United Kingdom	0	0	0	0	0	16	16
<b>Total by year</b>	<b>22</b>	<b>21</b>	<b>12</b>	<b>38</b>	<b>10</b>	<b>25</b>	<b>128</b>

**Table 3.** Tests and indicators<sup>a</sup> used for indexing *Prunus* fruit types.

Type of test/indicator	Almond	Apricot	Cherry	Peach	Plum
Graft transmission assays in a greenhouse <sup>b</sup>					
GF305 peach seedling	+	+	+	+	+
Kwanzan cherry	-	+	+	+	+
Peerless almond	+	-	-	-	-
Shiro plum	-	-	+	+	+
Shirofugen cherry	+	+	+	+	+
Tilton apricot	-	+	-	-	-
Graft transmission assay in an orchard <sup>c</sup>					
Bing cherry	-	-	+	+	-
Elberta peach	+	+	+	+	+
Luizet apricot	-	+	-	-	-
Moorpark apricot	-	+	-	-	-
Sam cherry	-	-	+	+	-
Herbaceous bioassays	+	-	+	+	+
ELISA (enzyme-linked immunosorbent assay)	+	+	+	+	+

<sup>a</sup> +/- indicates test is/is not applicable to fruit type. <sup>b</sup> Tests are replicated on three plants, once in each of two years. <sup>c</sup> Tests are performed on three indicator plants and observed through two fruit crops.

**Table 4.** Tests and indicators<sup>a</sup> used for indexing pome fruit genera (*Malus*, *Chaenomeles*, *Cydonia*, *Pyrus* spp.).

Type of test/indicator	Apple	Pear	Quince
Nucleic acid hybridisation	+	+	+
Graft transmission assays in a greenhouse <sup>b</sup>			
Radiant crab apple	+	-	-
Russian seedling R127407A	+	-	-
Virginia crab apple	+	+	+
Beurre Hardy pear	-	+	+
Nouveau Poiteau pear	-	+	+
Graft transmission assays in the growth chamber <sup>b</sup>			
Stark's earliest crab apple	+	+	+
Graft transmission assays in the orchard <sup>c</sup>			
Golden delicious apple	+	+	+
Lord Lambourne apple	+	+	+
Bartlett pear	-	+	+
Beurre Bosc pear	-	+	+
Beurre Hardy pear	-	-	+
Quince C7/1	-	-	+

<sup>a</sup> +/- indicates test is/is not applicable to fruit type. <sup>b</sup> Tests are replicated on three plants, one in each of two years. <sup>c</sup> Tests are performed on three indicator plants and observed through two fruit crops.

negative. Pome fruit field tests are begun when one year of greenhouse tests are negative.

### Indexing Protocols

Many diseases have been reported in deciduous fruit trees — as many as 60 in *Prunus* spp. However, the causal agents of only a few are known. Diagnosis is complicated by the fact that many diseases, such as apple green crinkle and russet ring and cherry rough fruit, induce symptoms on only the fruit of sensitive varieties. Consequently, lengthy orchard indexing processes that are labour and land intensive, environmentally unfriendly (requiring numerous pesticide applications) and expensive are still an integral part of the indexing process.

The indicators used in graft transmission tests and the supplemental laboratory tests used for stone and pome fruits are summarised by fruit type in Tables 3 and 4, respectively. The indexing relies primarily on graft transmission trials to woody indicator plants. Originally all assays were performed in orchard. The quarantine unit of NGRL has replaced many of the field tests with more rapid graft transmission tests that can be performed during winter months in a greenhouse or growth chamber. Symptoms usually appear on the indicator hosts in 2–3 months. The imported bud sticks may be used directly to perform tests while concurrently establishing propagations of the accession. However, negative results have been obtained when testing imported pear bud sticks and positive results have been obtained when testing the trees propagated from the bud sticks.

ELISA (enzyme-linked immunosorbent assay) and mechanical transmission tests to herbaceous tests are used in *Prunus* testing, but neither is reliable for pome testing. Electron microscopy has not expedited pathogen detection in either of these fruit groups.

Nucleic acid hybridisation assays that employ digoxigenin-labelled cRNA probes followed by detection using chemiluminescence have provided rapid assays for apple scar skin-like viroids in the pome fruits (Podleckis et al. 1993). The probes are used with tissue imprints made to positively charged nylon membranes. The reliability of the test is enhanced when the quarantined accession is first graft inoculated to a bioamplification host (host that gives a high titre, without symptoms) which is then sliced and used to make tissue imprints for the hybridisation assay. Inoculation of a common bioamplification host is also useful in overcoming the problem of inadequate positive and negative controls for the unique genotypes encountered in quarantine indexing programs. A similar approach is under investigation for pear blister canker viroid detection.

Phytoplasmas cause several diseases in these fruit groups (for example, apple proliferation [AP], pear decline, apricot chlorotic leaf roll) and are possible

causes of others (for example, flat limb/rubbery wood, chat fruit). In collaboration with R.E. Davis (Research Leader, Molecular Plant Pathology Laboratory, ARS, Beltsville, MD) polymerase chain reaction (PCR) technology is being applied to detect AP phytoplasma. Total nucleic acid extracted from petioles and midveins of AP-infected and healthy apple and periwinkle (*Catharanthus roseus* [L.] G. Don) were used as DNA template in PCR-mediated DNA amplification. Amplification was primed by an oligonucleotide pair (R16F2/R16R2), which gives amplification of a 1.2 kbp fragment of the 16S rRNA gene from all known phytoplasmas (Podleckis et al. 1993). However, the reaction may yield insufficient phytoplasma-specific DNA product for detection in agarose gel electrophoretic analysis. A more sensitive assay resulted when an oligonucleotide pair of primers nested within the positions of the universal primers was used for PCR with DNA product from the universal primer amplification as template. The nested primer pair (kindly provided by D.E. Gundersen and I.-M. Lee, MPPL) primes specific amplification of 16S rDNA from AP phytoplasma. Gel electrophoretic analysis of product from the second nested PCR revealed amplification of an AP characteristic DNA fragment in samples from AP-infected apple and periwinkle, but not healthy controls. Restriction fragment length polymorphism analysis of the products confirmed the identity of detected phytoplasmas as strains in group 16SSrX (AP and related strains). The enhanced detection of phytoplasma DNA by nested PCR should aid the detection of a broad spectrum of phytoplasmas in fruit tree, as well as other plant germplasm, if use of different phytoplasma group-specific primers are used in nested PCR mixtures (Lee et al. 1993).

### Incidence of Infection

The incidences of infections detected in the pome and stone fruits are very different. In testing 500 stone fruit accessions over six years, viral-like pathogens were found in less than 10% of the plants. The most commonly detected viruses are prune dwarf and prunus necrotic ringspot ilarviruses. Each was found (in single or mixed infections) in about 6% of the tested germplasm. Both viruses are detected easily by ELISA and herbaceous bioassays. Green ring mottle virus and plum pox potyvirus were detected in 7 and 9 accessions respectively. These viruses are also detected quickly in greenhouse graft transmission tests. Unidentified virus-like agents were detected in about 1% of other accessions.

In contrast to the low incidence of submicroscopic pathogens in stone fruit, the incidence of such pathogens in pome fruits is 60–70%. For example, 79 of 126 apples and 120 of 171 pears were positive to one or more viruses. Apples typically are infected by one or more of three 'common' or 'latent' viruses that include

apple chlorotic leaf spot trichovirus, stem pitting virus, and stem grooving capillovirus. Pear and quince are typically infected with mixtures of pear vein yellows virus, apple chlorotic leaf spot trichovirus, and stem grooving capillovirus. Apple mosaic ilarvirus and flat limb/rubbery wood agent(s) occur regularly in germplasm imported from several countries. Apple scar skin-like viroid was detected in 14 of 35 oriental pears.

The indexing procedures are managed differently as a result of the differences in infection incidence between pome and stone fruit trees. For example, since few stone fruit accessions are infected, multiple accessions are simultaneously inoculated into a single indicator tree. Pome fruits typically are tested individually — one accession on any one indicator tree. This results in large, orchard indexing blocks for pome fruit tree accessions.

Since so many pome fruits are infected, a therapy program for eliminating the pathogens was implemented. The program complicates the pome fruit tree quarantine in several ways:

- it adds 1–3 years to the quarantine period;
- it requires the use of growth chambers that can be expensive to purchase and maintain;
- it increases the inventory of trees that must be maintained and tracked; and
- it increases the indexing burden several fold because several heat treated propagants are indexed concurrently to ensure that at least one healthy tree will be detected.

Studying the etiology of fruit tree diseases with unknown causal agents is a slow process because the development of detection and assay methods can take years. The common occurrence of multiple infections in pome fruits compounds the problem of developing rapid bioassays and/or isolating and characterising the causal agents of the deleterious diseases. Without any knowledge of the biochemistry of the pathogens, the use of molecular technologies for their detection in either the pome or the stone fruit trees will be difficult. The quarantine of fruit tree germplasm, therefore, will be a long and complex process for several years.

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# Detection of Latent Viruses and Double-Stranded RNAs in Clonal Grasses During Post-Entry Quarantine

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## Abstract

Eight cultivars, comprising 18% of clonal grasses tested during post-entry quarantine at the Plant Quarantine Station, Rydalmere, were found to be infected by virus. Five infected cultivars were detected only by dsRNA analysis. Cultivars infected with potyviruses were not reliably detected with dsRNA analysis, but were reliably detected using mechanical inoculation to sweet corn cv. Supagold.

REQUESTS FOR IMPORTS of high risk, vegetatively propagated clonal grasses prompted the Australian Quarantine and Inspection Service (AQIS) to investigate the risks of introducing diseases with these grasses. A review prepared by Jones (1984) identified Pierce's disease bacterium (*Xylella fastidiosa*) and a number of exotic viruses of the Poaceae as quarantine risks. It was considered essential for the safe introduction of clonal grasses to Australia that a non-specific test be developed to detect viruses in these grasses.

In 1986 AQIS introduced a post-entry quarantine protocol to screen introductions of clonal grasses for disease (Australian Quarantine and Inspection Service 1986). Only grasses from countries free of maize streak virus (that is, New World countries) were permitted entry. Imported grasses were established in post-entry quarantine and inspected for disease symptoms every two weeks during the initial stage of growth. Then a sample of each clone was hot water treated at 50°C for two hours to eliminate *Xylella fastidiosa*. These treated plants were indexed by sap inoculation on sweet corn and tested for RNA viruses by a double-stranded RNA (dsRNA) test. The dsRNA test has been used to detect several viruses of the Poaceae (Reddy et al. 1975; Morris and Dodds 1979; Gildow et al. 1983; Mackie et al. 1988) as well as viruses from many other virus groups and hosts (reviewed in Dodds et al. 1984, Dodds 1986 and Jones 1992).

Dodman (1992) reviewed the risks posed by the introduction of clonal grasses, following the identification of problems with the effectiveness of the protocol. Deficiencies identified in this review led to the inclusion of a quick-dip electron microscopy test and mechanical inoculation to *Zea mays* cv. Supagold, *Triticum aestivum* cv. Hartog, and *Hordeum vulgare* cv. Tallon. These changes, as well as specific tests for maize chlorotic dwarf machlovirus and wheat dwarf

geminivirus, a phytosanitary declaration of freedom from Bermuda grass etched-line marafivirus and the exclusion of grasses from Africa and Asia, were included in a new protocol circulated by AQIS for comment in 1993 (Dodman 1993). Currently, 52 clones at various stages of screening are being held at the Plant Quarantine Station, Rydalmere, New South Wales, pending the acceptance of this protocol.

This paper sets out the results of screening tests on clonal grasses introduced through post-entry quarantine at Rydalmere, with selected reference to Indooroopilly, Queensland, and their implications for the testing of these grasses in the future.

## Experimental

### Electron microscopy quick dips (TEM-Qd)

Plant sap quick dips were prepared on 300 mesh copper grids coated with Parlodion<sup>®</sup> and carbon negatively stained with 2% w/v ammonium molybdate. These were examined with a Phillips EM 300 transmission electron microscope.

No quick dips were positive when prepared directly from clonal grasses. Potyvirus infections were identified from inoculated sweet corn (see below).

### Mechanical inoculations

Grass tissue was ground in 0.03 M phosphate buffer (potassium dihydrogen orthophosphate, disodium hydrogen orthophosphate) pH 7 with 2% polyvinylpyrrolidone and applied to indicator plants with carborundum abrasive (500 mesh). *Zea mays* cv. Supagold seedlings were inoculated at the 3–4 leaf stage with 4–5 plants per test. *Triticum aestivum* cv. Hartog seedlings and *Hordeum vulgare* cv. Tallon seedlings were inoculated at the 4–5 leaf stage with 6–8 plants per test.

*Zea mays* cv. Supagold was selected as the test cultivar when it was found that cultivars of sweet corn showed differing degrees of symptom expression

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(Singh 1990). Dodman (1992) reported that cv. Supagold showed the most reliable symptoms when inoculated with virus infected *Cynodon dactylon* A22 and *C. dactylon* E29. Tests at Rydalmere also showed that cv. Supagold produced stronger symptoms than the cvs. Honey Sweet and Iochief when inoculated with the *C. dactylon* E29 and *Miscanthus sinensis* cv. Morning Light.

Three clonal grasses with potyvirus infections were detected using mechanical inoculation to sweet corn seedlings. No positive results were recorded using mechanical inoculation to wheat or barley indicator seedlings.

### Double-stranded RNA extraction

Samples of at least 20 g of glasshouse grown clonal grasses, consisting of young and middle age leaf tissue, were harvested, avoiding senescent and dead tissues. Each sample was divided into approximately 10 g sub-samples and ground to a fine powder in liquid nitrogen. DsRNA was extracted from ground material using the CF-11 cellulose chromatography procedure of Gillings et al. (1993a) developed from methods published by Morris and Dodds (1979), Jordan et al. (1983) and Valverde et al. (1986), modified to allow for larger amounts of tissue. To improve the separation of higher molecular weight dsRNAs and reduce DNA contamination in some samples 0.75% agarose gels and a second cycle of CF-11 chromatography were included (Dodds and Bar-Joseph 1982; Jones 1992). The second cycle of CF-11 chromatography using a centrifugation method was carried out in an eppendorf tube (Gillings et al. 1993b).

DsRNA pellets were resuspended in 30 µl TE buffer, and electrophoresed in 1.2% or 0.75% agarose gels in TBE buffer pH 8.3 at 90V for 1.5–2.0 hours. The gel was stained in 0.5 µg/mL ethidium bromide, and photographed on a UV light box using an orange filter and Polaroid type 55 black and white film. DsRNA bands were then compared with positive controls and molecular weight markers, which were one or more of the following: barley yellow dwarf luteovirus, rice dwarf reovirus, citrus tristeza closterovirus, tobacco mosaic tobamovirus, cucumber mosaic cucumovirus and tomato mosaic tobamovirus.

Any discrete dsRNA band in the viral molecular weight range (approximately 1.5–20 kb) was presumed to be of viral origin. No attempt was made to identify viruses or virus groups, as this would have required additional testing.

Comparisons can be made between detection techniques for those clonal grasses presumed to be virus infected (Table 1). Two clones showed symptoms during post-entry quarantine. Infection of *Vetiveria zizanioides* was confirmed by dsRNA analysis. *Cynodon dactylon* E29 mechanically inoculated to sweet corn cv. Supagold revealed a potyvirus, which had been reported earlier from this clone and *C. dactylon* A22 at the Plant Quarantine Station, Eagle Farm, Queensland (Dr R.L. Dodman, Department of Primary Industries, Meiers Road, Indooroopilly, Queensland, personal communication). DsRNA analysis of *C. dactylon* E29 tissue did not confirm the viral infection. *M. sinensis* cv. Morning Light, a variegated variety, has not expressed symptoms. When sweet corn seedlings were inoculated, a systemic mosaic reaction

**Table 1.** Indexing results for infected clonal grasses.

Type/cultivar	Origin	TEM-Qd	Sweet corn cv. Supagold	Wheat cv. Hartog	Barley cv. Tallon	dsRNA	Visual
<i>Vetiveria zizanioides</i>	India	-	-	nd	nd	+	+
<i>Cynodon dactylon</i> E29	USA	-	+	- <sup>a</sup>	- <sup>a</sup>	-	+
<i>Miscanthus sinensis</i> cv. Malepartus	Germany	-	-	-	-	+	-
<i>Miscanthus sinensis</i> cv. Kaskade	Germany	-	-	-	-	+	-
<i>Miscanthus sinensis</i> cv. Kleine Fontane	Germany	-	-	-	-	+	-
<i>Miscanthus sinensis</i> var strictus	UK	-	-	-	-	+	-
<i>Miscanthus sinensis</i> cv. Morning Light	USA	-	+	-	-	+	-
<i>Molinia arundinacea</i> cv. Transparent	USA	-	-	-	-	+	-

<sup>a</sup> Test results from Dr R.L. Dodman, Department of Primary Industries, Meiers Road, Indooroopilly, Queensland, Australia.  
nd Not done.

**Table 2.** Detection of virus isolates of the potyvirus group.

Technique	<i>Cynodon dactylon</i> A22 <sup>a</sup>	<i>Cynodon dactylon</i> E29	<i>Miscanthus sinensis</i> cv. Morning Light
Visual screening	mosaic symptoms <sup>b</sup>	mosaic symptoms	- (variegated variety)
DsRNA, Indooroopilly <sup>b</sup>	-	-	nd
DsRNA, Rydalmere	- (24 g 20/10/92) <sup>c</sup> - (35 g 2/12/92)	- (20 g 3/3/92)	- (20 g 21/2/92) + (38 g 8/12/92) - (40 g 23/5/94) - (40 g 31/5/94)
Indexing on <i>Zea mays</i> cv. Supagold	+ <sup>b</sup>	+	+
TEM quick dip	- <sup>b</sup>	-	-
TEM quick dip of infected <i>Zea mays</i> cv. Supagold	+ <sup>b</sup>	+	+
DsRNA of infected <i>Zea mays</i> cv. Supagold	nd	+ (28 g 8/12/92)	+ (28 g 8/12/92)

<sup>a</sup> Held at Plant Quarantine Station, Eagle Farm, Queensland, Australia. <sup>b</sup> Test results from Dr R.L. Dodman, Department of Primary Industries, Meiers Road, Indooroopilly, Queensland, Australia. <sup>c</sup> Weight in grams of host tissue extracted and date. nd Not done.

occurred. Potyvirus particles were seen in a TEM quick dip. Of these eight infected lines, which represent 18.2% of the total number of clones tested so far, five were detected by the presence of dsRNA alone.

The dsRNA analysis, while giving positive results for a number of clonal grasses, failed to identify three potyvirus isolates in other clonal grasses (Table 2). However, positive results were obtainable from the inoculated sweet corn hosts using a minimum of 28 g of tissue. On one occasion the dsRNA analysis was positive for *M. sinensis* cv. Morning Light after 38 g of host tissue were extracted in early summer, but failed to repeat this result in two further tests during autumn.

### Discussion

The results of the testing program support the routine use of dsRNA testing in indexing programs (Dodds 1986). It also has a distinct advantage for plants with diseases of presumed viral etiology, where particles have yet to be identified (Morris and Dodds 1979; Jordan et al. 1983; Watkins et al. 1990). All of the grasses (Table 1) positive by dsRNA, yet negative by TEM and mechanical inoculation, are potential examples of this phenomenon. Unfortunately the dsRNA detection methods used in this paper could not routinely detect potyviruses. Only inoculations to a sweet corn

cv. Supagold avoided the release of three infected lines, and as yet it is not known how susceptible the wheat and barley indicators are to unknown viruses.

The recovery of dsRNA from some hosts can present a problem, because the optimal amount of tissue required for success is as much as 30 g (Valverde et al. 1986; Spiegel 1987; Mackie et al. 1988). Valverde et al. (1986) found that there was generally a low dsRNA yield for potyviruses and considered it related to the low titre of these viruses in their hosts. Up to 30 g of tissue were required for potyviruses to give comparable results to other viruses that required less than 7 g. For clonal grasses these amounts of tissue are likely to be even greater than those reported previously. Obtaining enough tissue for extraction of dsRNA from some clonal grasses required extra multiplication in post-entry quarantine.

The diversity of growth habits and environmental adaptations of the Poaceae may also be a limiting factor in visual and dsRNA methods of disease screening. The presence of variegations, other natural colour pigments and adaptations such as narrow leaves, can frequently interfere with observations for virus symptoms. The reliability of dsRNA analysis may also vary with the particular host and virus combination (Dodds et al. 1987; Mackie et al. 1988).

The reliability of virus detection using dsRNA from clonal grasses needs further study. Anomalies in

dsRNA tests that occur in some hosts (Jones 1992) or are produced by external insect or fungal contamination (Morris and Dodds 1979; Mackie et al. 1988) have not been examined in detail for the clonal grasses. In some hosts leaf tissues are suitable for dsRNA testing, but in others higher yields may be obtained from different plant tissue or these other tissues may be the only suitable dsRNA source (Jordan et al. 1983; Mirkov and Dodds 1985; Mackie et al. 1988).

It may be possible to produce consistent dsRNA results by collecting the optimum quantity of tissue at the correct growth stage, produced under the correct growing conditions (Dodds et al. 1987). These conditions have not yet been defined for clonal grasses. Dodman (1993) records more than 90 viruses capable of infecting clonal grasses, of which 7 are known to have DNA genomes and 6 are undetermined. Therefore, to supplement the current indexing, specific tests or geographic exclusions are required for DNA viruses and some RNA viruses considered to be of economic importance.

It is important to continue to monitor the health of introduced clonal grasses. In this way experience will be gained with dsRNA analysis of these grasses and its reliability in detecting virus infections. At present not enough is known about the titres of dsRNA and virus particles, or their replication in the grasses. As a result, absolute reliability of detection cannot be guaranteed by any individual testing method.

For quarantine testing to reliably detect all viruses in clonal grasses, further protocol development is necessary. The inclusion of additional specific tests in the quarantine protocol for clonal grasses will produce a functional, practical protocol that can be used to actively screen high risk clonal grasses. A total ban on their importation would run the risk of encouraging smuggling.

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# **Major Quarantinable Diseases of Sugarcane**

# Pest Risk Analysis and Its Implications for Pest and Disease Exclusion in International Germplasm Exchange

S. Singh\*

## Abstract

Pest risk analysis is essential for importing countries to develop or review their phytosanitary regulations and to ensure that regulatory pest control measures are justifiable and the least restrictive to international trade. It consists of two main parts — pest risk assessment and pest risk management. The former deals with identification and characterisation of quarantine pests and the latter with selection of appropriate, technically justifiable management options for mitigating the risks of their introduction into, and establishment in, an endangered area. The pest risk analysis process is resource demanding and highly dependent on international cooperation, particularly in instances where the available scientific and technical information is insufficient for a regulatory authority to reach a sound decision on the importation of a commodity. Pest risk analysis is a powerful tool for identifying gaps in current scientific knowledge and providing direction for investigations, including scientific research, required to support the removal of unnecessary impediments to the international exchange of plants, plant products and occasionally pests. Pest risk analysis offers tremendous opportunities for facilitating the international exchange of germplasm in an environment where plant industries increasingly require rapid access to germplasm, including commercial quantities, in a cost-effective and efficient manner, and potentially to minimise the risk of illicit trade.

PEST RISK ANALYSIS is about identifying pests of quarantine concern to an area, determining pathways and the likelihood of their introduction and establishment, and selecting optimum effective pest management options for minimising their introduction and establishment potential. The process of pest risk analysis is described later.

In this paper, pest includes disease and means 'Any species, strain or biotype of plant or animal, or any pathogenic agent, injurious to plants or plant products' (as defined in FAO 1995b), unless otherwise specified.

Quarantine pest in this paper means 'A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled' (as defined in FAO 1995b, subject to revision of the IPPC), unless otherwise specified.

## Why Pest Risk Analysis?

Under the International Plant Protection Convention (IPPC), the contracting parties, about 100 countries,

have agreed to adopt phytosanitary measures to prevent the introduction of quarantine pests<sup>1</sup> to endangered areas and that these measures should be the least restrictive to international trade (FAO 1992, 1995a). Further, the World Trade Organisation (WTO), formerly the General Agreement on Tariffs and Trade (GATT) under the GATT Agreement on the Application of Sanitary and Phytosanitary Measures (GATT 1994) requires that contracting parties not use unjustifiable phytosanitary measures as barriers to international trade. To comply with the spirit of these IPPC and WTO agreements, the contracting parties have little option but to use pest risk analysis in their decision making process, which must be transparent, for the development of an importation policy for plants and plant parts.

With the aim of developing international plant quarantine standards, the Secretariat of the IPPC has identified 16 principles of plant quarantine (FAO 1995a) consistent with the intentions of the IPPC. The need for a structured pest risk analysis framework results from the implementation of the principle of risk analysis at a practical level and consideration of other principles, namely managed risk, necessity, and minimal impact. Other key principles that affect pest risk analysis are transparency and harmonisation.

## Principle of risk analysis

To determine which pests are quarantine pests and the strength of the measures to be taken against them, countries shall use pest risk analysis methods based on

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<sup>1</sup> In this instance quarantine pest means 'Any form of plant or animal life, or any pathogenic agents of potential national economic importance to the country endangered thereby and not yet present there, or present but not widely distributed and being actively controlled' (as referred in FAO 1992).

biological and economic evidence and, wherever possible, follow procedures developed within the framework of the IPPC.

### **Principle of managed risk**

Because some risk of the introduction of a quarantine pest always exists, countries shall agree to a policy of risk management when formulating phytosanitary measures.

### **Principle of necessity**

Countries shall institute restrictive measures only where such measures are made necessary by phytosanitary considerations, to prevent the introduction of quarantine pests.

### **Principle of minimal impact**

Phytosanitary measures shall be consistent with the pest risk involved and shall represent the least restrictive measures available that minimise the impediment to the international movement of people, commodities and conveyances.

### **Principle of transparency**

Countries shall publish and disseminate phytosanitary prohibitions, restrictions and requirements and make available, on request, the rationale for such measures. The key here is the 'rationale' — the pest risk analysis that has been conducted to develop phytosanitary measures to minimise the quarantine pest risks.

### **Principle of harmonisation**

Phytosanitary measures shall be based, whenever possible, on international standards, guidelines and recommendations, developed within the framework of the IPPC. International standards include the procedures for conducting pest risk analysis at a national level, as well as other relevant concepts, including a glossary of terms (FAO 1990).

## **When Is Pest Risk Analysis Required?**

Pest risk analyses of various complexities are required in most plant quarantine import situations, and are particularly relevant in the international exchange of germplasm, predominantly in the following situations:

- to consider the importation of a plant species for the first time;
- to evaluate a new method for the importation of a plant species;
- to consider a new source of origin, including pest-free areas, for a plant species already being imported from some other source;

- to monitor advances in technology — for example, the availability of new detection techniques and pest management options for mitigating the risks of introducing a quarantine pest;
- to review existing phytosanitary restrictions by a national plant protection organisation<sup>2</sup>, following, for example, a challenge from clients and/or overseas trading partners, or realisation of inadequacies in the current phytosanitary measures;
- to evaluate a new economically important pest described overseas and the risk it presents to existing or potential trade of the importing country — for example, the detection of banana bract mosaic virus in the Philippines led to amendments to Australia's importation policy for banana germplasm;
- to evaluate options when a new pathway, including an alternative or a collateral host plant species, for the introducing a quarantine pest is discovered — for example, Australia reviewed the importation policy for alternative hosts of the Moko disease bacterium following its detection in 1989 in heliconia plants imported from Hawaii; and
- to consider a request for the importation of a pest for research work in a controlled environment — for example, for use as a positive control in diagnostic tests or for testing efficacy of pest management options.

Pest risk analysis would be a useful tool for WTO expert panels while reviewing phytosanitary conditions for the purpose of settling potential disputes under the GATT sanitary and phytosanitary agreement.

## **How Is Pest Risk Analysis Conducted?**

Various forms of pest risk analysis have been conducted since the introduction of quarantine measures. Most of the pest risk analysis work for determining the quarantine status of pests has been conducted following the IPPC definition, which has been the subject of considerable debate (Phillips et al. 1994). In Australia the use of risk analysis was formally identified by the Lindsay review committee (Quarantine Review Committee 1988). After that it became government policy to accept risk analysis even before the development of the sanitary and phytosanitary agreement under the GATT.

A more structured approach to pest risk analysis has been necessitated by demands within the sanitary and phytosanitary agreement for transparency in decision making processes of national plant protection organisations, and because of its potential use as the key decision making process in the settlement of disputes where a country is using unjustifiable phytosanitary measures as barriers to trade. The FAO has drafted a standard for pest risk analysis to support the IPPC mandate as it relates to international trade in plants and plant products (FAO 1995b). It is anticipated that the draft standard will be ratified by the FAO conference in November 1995. The standard is a significant advance

<sup>2</sup> A national plant protection organisation in this paper means an official service established by a government to discharge the functions specified by the IPPC (as defined in FAO 1990).

toward providing an internationally accepted framework for conducting structured pest risk analysis. Its salient features are:

- the plant quarantine terms used in the pest risk analysis are well defined; and
- the process of pest risk analysis (and its subprocesses of pest risk initiation, pest risk assessment and pest risk management) is described in detail.

Over the years a simple pest risk analysis model, which is consistent with the spirit of the IPPC and the draft FAO standard, has been used in advising the Australian Quarantine and Inspection Service on the formulation of importation policy for various plants and plant products (Singh 1991, 1994, 1995a,b,c; Singh and Paton 1991). Figures 1 and 2 show the steps followed in pest risk analysis work on any nominated commodity and pest, respectively. Since the information on biology and economic consequences of pests is often patchy, particularly for pests of medium significance, it is necessary to rely on the best available information and professional judgments. In instances where available information has been insufficient, a somewhat conservative approach consistent with the Australian Government (1988) policy statement has been used, identifying the need for further investigation(s) to fill the gaps in the available scientific knowledge revealed by the pest risk analysis.

### Identification of Quarantine Pests

In addition to considering pest lists provided by proposed exporting countries, it is useful to conduct exhaustive investigations, including literature searches, and to communicate with scientists within Australia and overseas, on the pest status in the exporting countries and worldwide, with particular emphasis on the adjoining countries and the region. This type of verification is essential because the pest status in certain areas might not be fully investigated or adequately recorded.

The criteria that need to be satisfied before a pest is considered a quarantine pest are dealt with in detail in the draft FAO standard. Generally a pest risk analysis is conducted for access of a commodity with a background of legislation currently in force — that is, quarantine pests are listed. However, for new commodities the pest risk analysis may identify new pests of concern.

The FAO draft standard makes provision to consider a pest as a quarantine pest if it is under consideration for future official control but is not widely distributed and is of potential economic importance to the pest risk analysis area. Having not come across this situation in pest risk analysis work, this scenario is not included in Figure 2.

The economic importance criterion (including introduction and establishment potential) presents the most significant ongoing challenges for pest risk analysis work, although the taxonomy and the search for effective

but the least restrictive pest management options can be equally challenging.

### The Need for International Germplasm Exchange and the Associated Pest Risks

There is an increasing demand for the international movement of germplasm for many reasons, including:

- the demands of agricultural and horticultural industries, driven by national and international competitiveness imperatives, for plant varieties with improved quality, yield and pest resistance;
- the need for access to commercial quantities of superior quality propagation material being produced overseas because the environmental conditions and/or economic considerations in the importing country do not favour production of high quality propagation material;
- increasing interest in breeding new varieties due to plant breeders' rights and the application of molecular biology; and
- increased interest in collecting and preserving germplasm.

It is common knowledge that the introduction of infected or infested germplasm to an endangered area presents one of the greatest quarantine risks in international commodity trade. While most of the insect and mite risk concerns can currently be satisfactorily addressed by inspection and fumigation or insecticide treatments, the use of chemical treatments is increasingly subject to scrutiny for human health and environmental reasons.

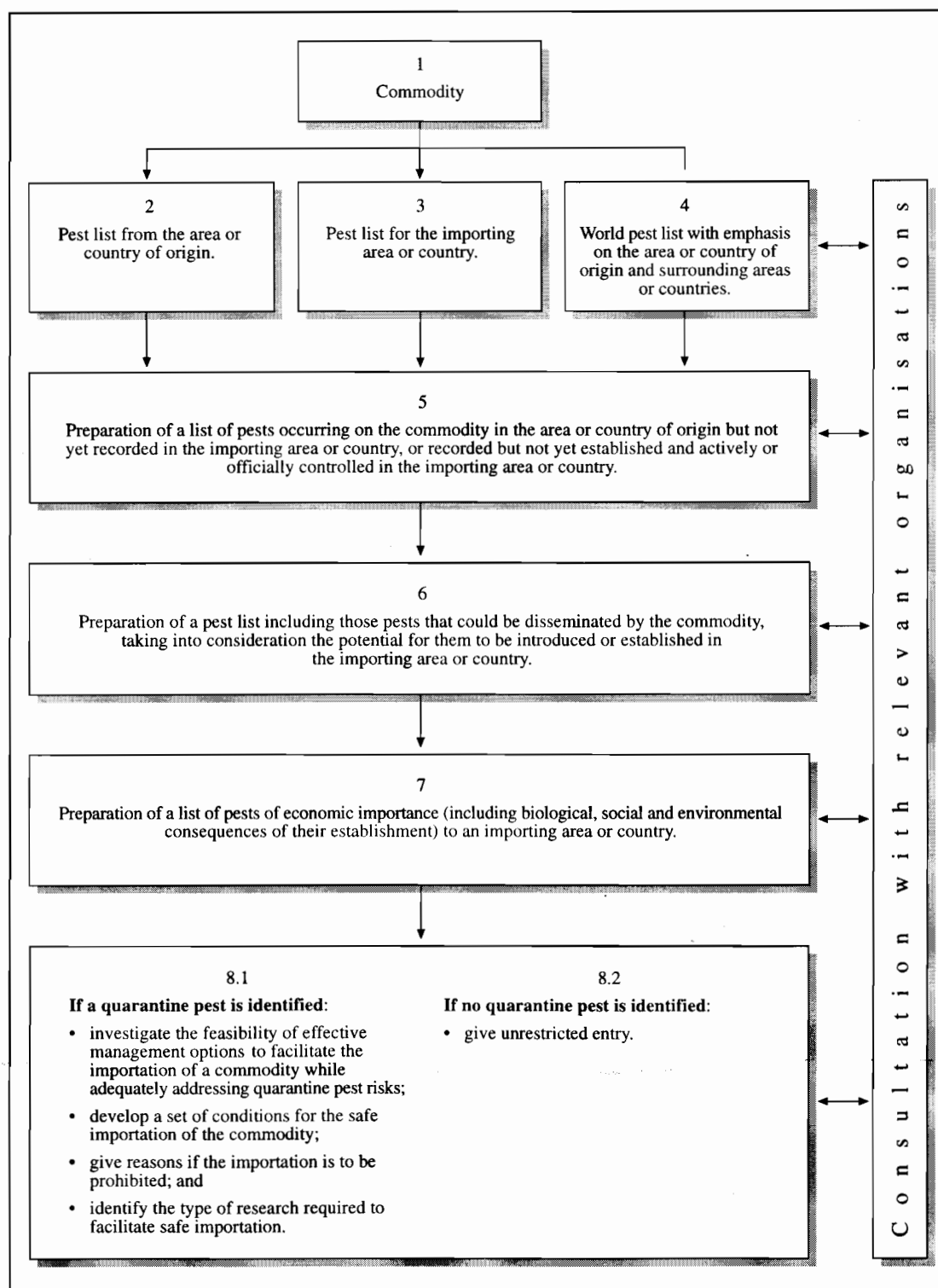
Plant disease risks by their nature are frequently difficult to address, particularly symptomless and systemic infection and surface contamination with live pathogens. A range of diseases of quarantine concern could be disseminated in, on or with germplasm and potentially have significant consequences for an endangered area. The diseases listed in Table 1 would have significant economic consequences for Australia if they were introduced with, among other things, imported vegetative propagation material and became established in this country.

### Implications of Pest Risk Analysis for International Exchange of Germplasm

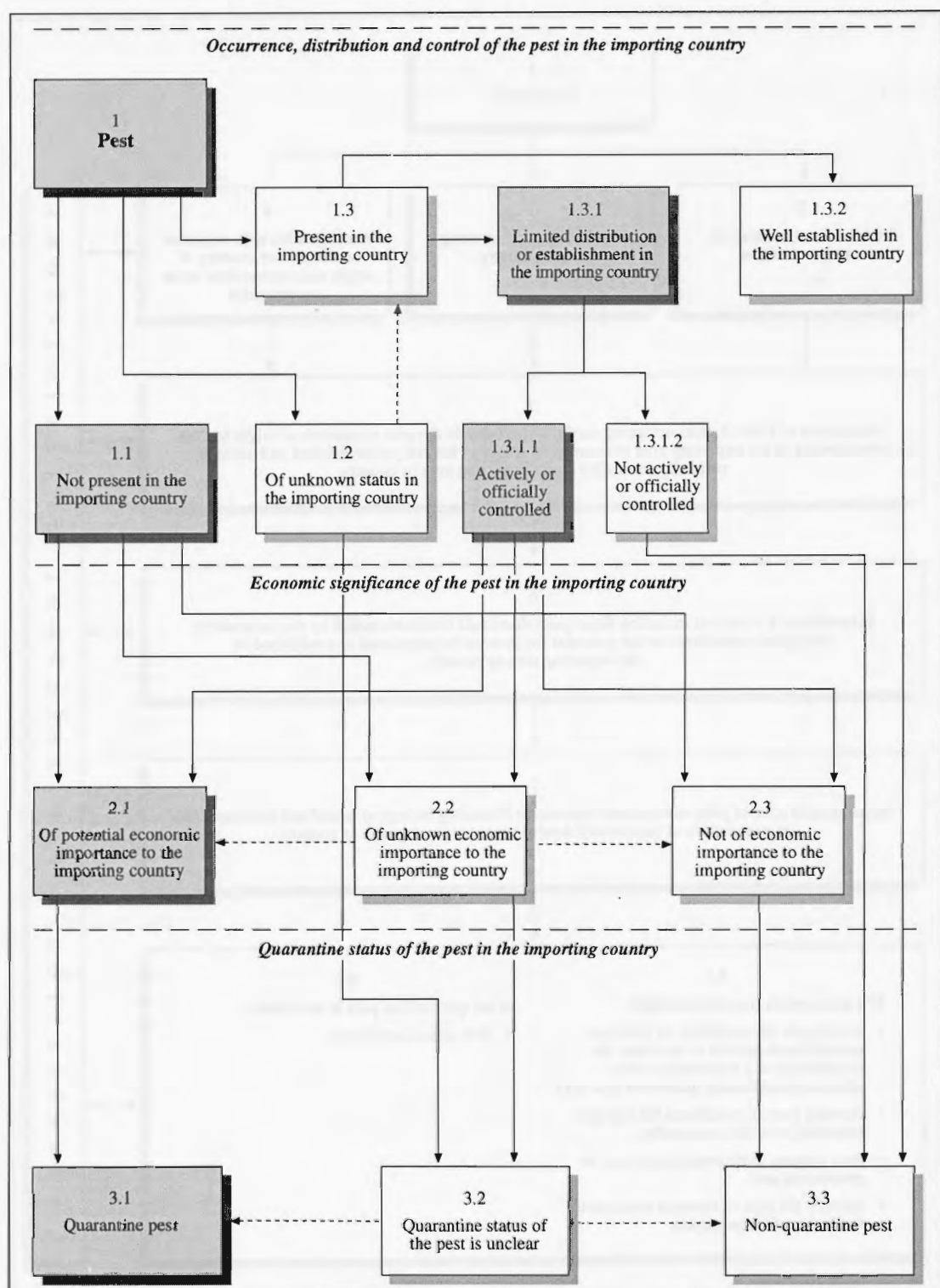
Pest risk analysis is an effective tool for identifying and targeting quarantine pests for the purpose of developing specific and justifiable phytosanitary measures. A thorough pest risk analysis would identify most available management options that can be used independently or in various combinations, if required, to minimise the perceived quarantine pest risks to acceptable levels.

By implementing the management options identified by pest risk analysis and facilitating trade in germplasm, there is less incentive for illicit trade in





**Figure 1.** A step-by-step schema for conducting pest risk analysis for a commodity.



**Figure 2.** A step-by-step schema for determining the quarantine status of a pest.

**Table 1.** Important diseases that may be introduced into Australia by importing, among other things, plants and vegetative plant parts in their natural state.

Pathogen	Disease	At risk
<b>Bacteria</b>		
Citrus greening bacterium?	Citrus greening	Citrus
<i>Clavibacter michiganensis</i> ssp. <i>sepedonicus</i>	Ring rot	Potato
<i>Endocronartium harkenssii</i>	Western gall rust	Pine
<i>Erwinia amylovora</i>	Fireblight	Pome fruit
<i>Pseudomonas solanacearum</i>	Bugtok disease	Banana
<i>Pseudomonas solanacearum</i> race 2	Moko disease	Banana
<i>Pseudomonas solanacearum</i> ? (syn. <i>Pseudomonas celebensis</i> )	Blood disease	Banana
<i>Pseudomonas syringae</i> pv. <i>savastanoi</i>	Olive knot	Olive
<i>Xanthomonas campestris</i> pv. <i>citri</i>	Citrus canker	Citrus
<i>Xanthomonas campestris</i> pv. <i>manihotis</i>	Cassava bacterial blight	Cassava
<i>Xanthomonas campestris</i> pv. <i>vasculorum</i>	Gumming	Sugarcane
<i>Xanthomonas fragariae</i>	Strawberry angular leaf spot	Strawberry
<i>Xylella fastidiosa</i>	Pierce's disease, citrus variegated chlorosis, plum leaf scald, almond leaf scorch	Grapevine, citrus, plum, almond
<i>Xylophilus ampelinus</i> (syn. <i>Xanthomonas ampelina</i> )	Bacterial blight	Grapevine
<b>Fungi</b>		
<i>Ceratocystis ulmi</i>	Dutch elm disease	Elm
<i>Blumeriella jaapii</i>	Cherry leaf spot	Cherry
<i>Chryphonectria parasitica</i> (syn. <i>Endothia parasitica</i> )	Chestnut blight	Chestnut
<i>Exobasidium vexans</i>	Blister blight	Tea
<i>Fusarium oxysporum</i> f.sp. <i>albedinis</i>	Bayoud disease	Palm
<i>Guignardia bidwellii</i>	Black rot	Grapevine
<i>Hemileia vastatrix</i>	Coffee leaf rust	Coffee
<i>Monilinia fructigena</i>	Brown rot	Pome fruit
<i>Nectria galligena</i>	European	Apple
<i>Peronosclerospora sacchari</i>	Sugarcane downy mildew	Sugarcane, maize
<i>Phoma tracheiphila</i> (syn. <i>Deuterophoma tracheiphila</i> )	Mal secco	Citrus
<i>Pseudoperonospora humuli</i>	Downy mildew	Hops
<i>Pseudopeziza tetraspora</i>	Angular leaf scorch	Grapevine
<i>Pseudopeziza tracheiphilla</i>	Rotbrenner	Grapevine
<i>Puccinia psidii</i>	Guava rust	Guava, eucalypt
<i>Synchytrium endobioticum</i>	Black wart	Potato
<i>Ustilago scitaminea</i>	Sugarcane smut	Sugarcane
<b>Nematodes</b>		
<i>Globodera</i> spp. ( <i>G. pallida</i> & <i>G. rostochiensis</i> )	Potato cyst nematode	Potato
<i>Heterodera glycines</i>	Soybean cyst nematode	Soybean
<i>Bursaphelenchus xylophilus</i>	Pinewood nematode	Pine
<b>Phytoplasmas</b>		
Coconut lethal yellowing	Lethal yellowing	Palms
Papaya bunchy top	Bunchy top	Papaw
Pear decline	Pear decline	Pear
Apple proliferation	Apple proliferation	Apple

(Continued on next page)

**Table 1.** (continued)

Pathogen	Disease	At risk
<b>Spiroplasma</b>		
<i>Spiroplasma citri</i>	Citrus stubborn	Citrus
<b>Viroids</b>		
Coconut cadang-cadang viroid	Cadang-cadang	Coconut
Potato spindle tuber viroid	Spindle tuber	Potato
<b>Viruses</b>		
Nepoviruses (several)	Various	Various
Papaya mosaic potexvirus	Papaw mosaic	Papaw
Peanut clump furovirus	Red leaf mottle, peanut clump	Sugarcane, peanut
Peanut stripe potyvirus	Peanut stripe	Peanut
Plum pox potyvirus	Plum pox, sharka	Stone fruit

germplasm and hence the quarantine risks arising from illegal activity are minimised.

International cooperation in the acceptance and application of pest risk analysis will in itself improve the knowledge base through information exchange for pest risk analysis practitioners and decision makers, and enhance the safety of international germplasm exchange.

Quality assurance systems for accrediting overseas sources can be built around the biological information and pest management options — for example, pest and disease screening and indexing generated by a pest risk analysis in order to facilitate importation of pest and disease free germplasm. In certain instances, depending on the risk involved and the availability of appropriate pest management options, commercial or semi-commercial quantities of germplasm may be imported into an otherwise endangered area. It is extremely important that any quality assurance arrangements are based on sound technical analysis and that there is appropriate infrastructure to ensure that the quarantine security of the importing country is not compromised. The FAO and International Board for Plant Genetic Resources technical guidelines for the safe movement of germplasm of many plant species have identified sources of known virus tested material. They would require evaluation, by national plant protection services, against the key quarantine pests from these guidelines and other relevant publications, before imports are considered. If circumstances change due to, for example, new developments in science and technology, then the current pest risk analysis may need to be reconsidered. Any amendments to such arrangements should be thoroughly assessed in consultation with experienced scientists because even minor changes could be crucial in preventing the introduction of a quarantine pest.

Since pest risk analysis will identify gaps in knowledge, it will promote research and development, par-

ticularly in the areas of pest diagnosis, efficacy testing and treatments for minimising or eliminating quarantine pest risks.

Certain importers — for example, germplasm resource centres — requiring germplasm imports free from non-quarantine pests will need to make independent arrangements, including obtaining overseas accreditation from a germplasm repository, for such imports as this is not the primary role of a quarantine regulatory authority. These arrangements should not be imposed, according to the draft FAO standard for pest risk analysis, on potential importers such as commercial propagators who do not wish to sacrifice time and money on non-quarantine pests.

The procedures for importing sugarcane germplasm into Australia have been developed to minimise the risk of introducing and establishing quarantine pests in this country. Australia's importation conditions for sugarcane are currently under review.

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# Sugarcane Bacilliform Virus, Sugarcane Mild Mosaic Virus and Sugarcane Yellow Leaf Syndrome

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## Abstract

Sugarcane bacilliform badnavirus (SCBV) occurs worldwide in practically all *Saccharum officinarum* clones, and occurs in *S. barberi*, *S. robustum*, *S. spontaneum*, *S. sinense*, *Saccharum* hybrids, *Rottboellia exaltata*, *Panicum maximum*, and *Sorghum halepense*. SCBV is transmitted by at least three species of mealy bug, and infects rice and banana. SCBV infection is associated with whitish-chlorotic leaf streaking in some *S. officinarum* clones, but is symptomless in the majority of cases. There is little information on the effects of SCBV infection on the yield of sugarcane. SCBV can be detected by enzyme-linked immunosorbent assay (ELISA), immunosorbent electron microscopy (ISEM) and polymerase chain reaction (PCR) amplification. Initial experiments indicate that heat treatment and meristem tip culture are ineffective in eliminating SCBV from *Saccharum* germplasm.

Sugarcane mild mosaic virus (SCMMV) is a mealy bug transmitted clostero-like virus that occurs in mixed natural infections with SCBV in *S. officinarum*, *S. robustum*, *S. sinense* and *Saccharum* hybrids. SCMMV infection occurs in some sugarcane cultivars with field growth disorders. SCMMV is detected most reliably by ISEM.

The form of yellow leaf syndrome (YLS) occurring in a number of commercial sugarcane cultivars in Brazil, Florida (USA) and Queensland (Australia) is associated with infection by an aphid transmitted luteovirus-like agent, which has been detected by electron microscopy, dsRNA analysis and PCR amplification. Symptoms of YLS include yellowing of the midrib and lamina. The variety SP71-6163 affected by YLS in Brazil shows significant decreases in sugar yield, but similar effects have not been documented elsewhere. It is possible that the form of YLS occurring in Hawaii may have a different cause.

## Sugarcane Bacilliform Virus

### Occurrence

SCBV was first detected in the *Saccharum* hybrid Mex. 57-473 in Morocco in 1986. The virus was subsequently found to occur worldwide in almost all clones of noble canes (*S. officinarum* L.) and in a fairly large number of commercial *Saccharum* hybrids. SCBV has also been identified in collections of *S. barberi*, *S. robustum*, *S. spontaneum* and *S. sinense*.

### Symptoms

Symptoms of SCBV infection in naturally infected *Saccharum officinarum* vary from no apparent foliar symptoms to pronounced whitish-chlorotic streaking. In one transmission experiment, an isolate of SCBV that produces pronounced symptoms in *S. officinarum*

produced mild transient chlorotic streaking in the clone CL61-620. In general, however, SCBV infection in commercial hybrids has not been associated with foliar symptoms.

### Taxonomy and virion properties

SCBV is a member of the plant virus genus Badnavirus, which belongs to the family Pararetroviridae, representing viruses containing a dsDNA genome that is replicated via reverse transcription. SCBV has non-enveloped bacilliform particles averaging 30 x 120–150 nm in size containing a 7.5 kb circular dsDNA genome that is interrupted by two site-specific discontinuities and contains three open reading frames (ORFs I–III) encoding proteins of 22, 13 and 215 kd, respectively. The virion capsid protein subunits have a molecular mass of 39 kd, but proteolysis by host plant enzymes results in the formation of a second 37 kd capsid protein species.

### Transmission

SCBV is transmitted both by vegetative propagation and by mealy bug vectors, which probably transmit the virus in a semipersistent manner, based on comparisons with cacao swollen shoot and *Commelina* yellow mottle badnaviruses. Three mealy bug species

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(Hemiptera, Pseudococcidae) have been shown experimentally to transmit SCBV. *Saccharicoccus sacchari*, the pink sugarcane mealy bug, and *Dysmicoccus boninensis*, the gray sugarcane mealy bug, transmitted SCBV from infected to healthy sugarcane, while *S. sacchari* and *Planococcus citri*, which does not readily colonise sugarcane, transmitted SCBV from sugarcane to banana.

SCBV can also be transmitted by mechanical inoculation using partially purified extracts. As in the case of other badnaviruses, it is considered unlikely that SCBV can be transmitted mechanically on cutting implements or machinery.

Several other badnaviruses have been shown to be seed and/or pollen transmitted. Given that noble canes (*Saccharum officinarum* L.) used in breeding programs are almost totally infected with SCBV, the important question of whether, and to what extent, seed and/or pollen transmission of SCBV occurs remains unanswered.

### Host range

SCBV occurs naturally in *S. officinarum*, *S. barberi*, *S. robustum*, *S. spontaneum*, *S. sinense*, *Saccharum* hybrids, *Sorghum halepense*, *Brachiaria* sp., *Panicum maximum* and *Rottboellia exaltata*, and has been transmitted experimentally to rice (*Oryza sativa*), banana (*Musa* AAA 'Dwarf Cavendish') and sorghum (*Sorghum vulgare* L.). When three isolates of SCBV that produce varying degrees of chlorotic streaking in naturally infected *S. officinarum* were transmitted by mealy bug to Dwarf Cavendish banana (*Musa* AAA), foliar symptoms very similar to those caused by banana streak virus developed, and the severity of symptoms exactly paralleled those occurring in the *S. officinarum* source plants.

SCBV is atypical among badnaviruses, having a relatively wide host range. Sugarcane is commonly grown close to other crops, both in commercial plantings as well as in home gardens, and the ability of the mealy bug vectors of SCBV to colonise a taxonomically diverse range of plants leads to the possibility of SCBV moving from sugarcane to new host plants. It is interesting to note that badnaviruses occurring in *Musa*, citrus, pineapple, *Canna* and *Kalanchoë* are all related serologically to SCBV.

### Effects of SCBV infection in sugarcane

SCBV infection occurs in practically all clones of *S. officinarum*, sometimes causing no apparent symptoms and sometimes producing chlorotic leaf streaking. To determine the effect of SCBV on the growth and yield of current commercial *Saccharum* hybrids, three commercial varieties, CP65-357, CP63-588 and CL61-620 were mechanically inoculated in a field experiment with a mixture of SCBV isolates from *S. officinarum*. Preliminary results indicate that SCBV infection in some cases adversely affects plant performance, and in

other cases has no measurable effect. It would be of interest to pursue this experiment to determine whether SCBV infection may have long term effects that are not initially apparent.

A second aspect of SCBV infection in sugarcane that warrants further investigation is the possibility of synergistic effects when SCBV infected clones become infected with other viruses. For example, in the *S. officinarum* clone Raratonga 1 the titre of SCBV and the severity of foliar symptoms increase dramatically when plants are also infected with sugarcane mosaic potyvirus. The varieties NCo376, N14, Waya and B41-227, affected by a growth disorder in Malawi, were found to be infected by a complex of viruses, including SCBV. It is possible that infection by SCBV alone or in combination with other viruses may render sugarcane more susceptible to other stress factors, resulting in symptom production and/or reduced plant performance.

### Detection of SCBV

SCBV infection in *Saccharum* germplasm can be detected by four methods, all of which have certain limitations.

#### Foliar symptoms

Some isolates of SCBV occurring in naturally infected *S. officinarum* clones (for example, NG57-239, Jamaica Red and Selemi Bali) cause well-defined whitish-chlorotic or chlorotic streak symptoms that are especially apparent in vigorously growing plants. However, in most clones of *S. officinarum* and other *Saccharum* species and hybrids, SCBV infection is not associated with foliar symptoms.

#### Electron microscopy

In some *S. officinarum* clones the concentration of SCBV virions is sufficiently high to allow detection by electron microscopic examination of crude leaf sap (leaf dips). In many *Saccharum* clones, however, it is necessary to prepare partially purified extracts from 5–10 g of leaf tissue (minipreps) in order to more reliably detect SCBV by examination using the electron microscope.

#### Serology

Reliable serological detection of SCBV is hindered by the existence of a wide degree of heterogeneity among naturally occurring isolates of the virus. To overcome this problem, polyclonal antisera have been raised against a mixture of SCBV isolates identified as being serologically distinct. These antisera detect a wide range of SCBV isolates in double-antibody sandwich ELISA (DAS-ELISA), and a wider range of isolates can be detected by indirect ELISA using F(ab')<sub>2</sub> fragments of IgG [F(ab')<sub>2</sub>-ELISA]. Currently, the most reliable method of SCBV indexing appears to be immunosorbent electron microscopy using partially purified minipreps.

### **Amplification by polymerase chain reaction**

Detection of SCBV by PCR amplification represents, in principle, a possible method for overcoming the barrier to reliable serological indexing caused by the extremely heterogeneous nature of SCBV antigens. Three degenerate oligonucleotide primers, based on consensus sequences of the tRNA<sup>Met</sup> binding site, and the reverse transcriptase and ribonuclease H coding regions, respectively, of several badnaviruses, have been shown to be capable of priming the amplification of 0.9 and 1.3 kb virus-specific products from genomic DNA of all badnaviruses except rice tungro bacilliform virus. However, two problems have so far prevented the application of PCR amplification, using these degenerate primers, to routine detection of SCBV. Firstly, nucleic acid preparations from sugarcane are frequently contaminated with polysaccharide, phenolic or other compounds that are potent inhibitors of Taq polymerase. Secondly, the genomic heterogeneity among SCBV isolates is as great as, or greater than, the serological heterogeneity that was described above. In effect, the SCBV isolate occurring in a given *Saccharum* clone does not represent a genomically homogeneous population, but a widely diverse mixture of genomic species. It is therefore perhaps not surprising that the degenerate badnavirus sequences described above do not reliably prime amplification of all SCBV isolates.

It has been recently reported that oligonucleotide sequences that prime amplification of smaller (200–400 bp) segments of the viral genome can reliably detect SCBV. Similar results have been obtained at the University of Minnesota, using sequences that prime the amplification of 300–450 bp products. However, certain experimental results question whether the PCR product is always SCBV-specific, and the use of these primers for routine indexing for SCBV is being approached with caution until further experiments are completed.

### **Elimination of SCBV from *Saccharum* germplasm**

Data from preliminary experiments using apical meristem culture with or without prior heat treatment (thermotherapy) indicate unequivocally that this method does not eliminate SCBV from progeny plants. On the contrary, progeny plantlets tended to contain higher concentrations of virions than did the source plants. Similar observations have been made with banana streak virus in *Musa* germplasm, suggesting that such conventional tissue culture based methods of pathogen elimination may be of no efficacy for the production of badnavirus-free germplasm.

Badnaviruses replicate their genome via reverse transcription, a process that is not essential to the normal functioning of eukaryotic cells. Chemotherapeutic methods based on specific inhibition of viral reverse transcriptase, without deleterious ancillary effects on the plant, followed by meristem tip culture,

may represent one possible approach to eliminating SCBV infection from germplasm collections of *S. officinarum* and other *Saccharum* and related species and hybrids used in sugarcane breeding programs.

## **Sugarcane Mild Mosaic Virus**

### **Distribution and occurrence**

Sugarcane mild mosaic virus has been identified in *S. officinarum*, *S. barberi*, *S. sinense* and a number of commercial *Saccharum* hybrids from Mauritius, Malawi, Florida and Australia. SCMMV has always been found in mixed infections with SCBV, with which it shares a common mealy bug vector. No widespread surveys have been done to estimate the geographical distribution and frequency of occurrence of SCMMV.

### **Taxonomy and virion properties**

Virions of SCMMV are 1500–1600 nm in length and have the helical characteristics of closteroviruses. Based on these criteria SCMMV has been tentatively classified as a member of the genus *Closterovirus*. However, additional physicochemical data need to be generated in order to confirm the taxonomic identity of SCMMV.

### **Symptoms**

In naturally infected *Saccharum* spp. SCMMV has been found only in mixed infections with SCBV. SCMMV was separated from SCBV by passage through the clones IJ76-319 and IK76-69, which appear to support limited replication of the SCBV isolate occurring in the source plant. No symptoms have been observed in either IJ76-391 or IK76-69, but a very mild interveinal mosaic can occasionally be observed in young leaves of infected CL61-620.

### **Transmission**

SCMMV is transmitted in nature by vegetative propagation and very probably by the pink (*Saccharicoccus sacchari*) and gray (*Dysmicoccus bonisii*) sugarcane mealy bugs, which have been shown experimentally to transmit the virus. Given the low concentration of the virus in infected plants and the inefficiency of transmission using sap extracts, it is unlikely that SCMMV would be spread mechanically on tools and machinery.

### **Host range**

SCMMV occurs naturally in *S. officinarum*, *S. barberi*, *S. sinense* and a number of commercial *Saccharum* hybrids including M27-16, N-14, NC0376, B41-227 and Waya, and has been transmitted experimentally to only rice (*Oryza sativa*) and *Sorghum halepense* but not to a number of other cultivated and wild species in the Gramineae.



### Effects of SCMMV infection

There are no experimental data on sugarcane or field observations of the effects of SCMMV infection on the growth or yield of sugarcane. As mentioned above, it is of interest to note that in Malawi a disorder of the varieties NCo376, B41-227, N14 and Waya was associated with infection by both SCBV and SCMMV. It is possible that SCMMV, like SCBV, may affect sugarcane less by causing direct, observable damage than by rendering infected plants more prone to damage by other stress factors.

### Detection of SCMMV

SCMMV can be readily detected by ISEM using partially purified minipreps. The two antisera that have been raised against SCMMV are not suitable for routine ELISA testing because of unacceptably high background reaction, but give good results in ISEM. Improved purification methods that largely eliminate immunogenic host plant contaminants, particularly phytoferritin, have been developed for SCMMV, and future production of antisera suitable for ELISA testing is envisaged.

### Elimination of SCMMV from *Saccharum* germplasm

Various forms of thermotherapy, followed by meristem tip culture, have been used to eliminate other closteroviruses (for example, Citrus tristeza virus) from infected plants, and it is very possible that a similar procedure could be employed to produce SCMMV-free plant material. It is not known whether the hot water treatments normally used for pathogen control in sugarcane germplasm exchange have any effect on SCMMV.

## Sugarcane Yellow Leaf Syndrome

### Occurrence and symptoms

The disorder known as yellow leaf syndrome of sugarcane has been reported from Brazil, Florida, Queensland and Hawaii. Symptoms consist of yellowing leaves, although a more characteristic and diagnostic symptom is the development of strong yellow or pinkish colouration of the midrib, which often occurs while the lamina is still green.

### Effect of YLS on sugarcane

The most thoroughly documented studies of the effects of YLS on crop performance have been carried out in the sugarcane producing areas of São Paulo, where the widely grown cv. SP71-6163 has been found to be susceptible to YLS and to suffer losses of 40–60 per cent of recoverable sugar. A similar degree of symptom severity and percentage infection has not been reported elsewhere.

### Etiology of YLS

Field observations of patterns of YLS distribution in São Paulo led to the conclusion that the disease syndrome was probably due to a transmissible agent rather than to abiotic factors such as adverse soil or climatic conditions. Because of the yellow-type symptoms associated with YLS, a virus or virus-like pathogen was suspected to be the causal agent of the disorder. Electron microscopic examination of partially purified extracts of YLS-infected leaf tissue revealed the presence of low concentrations of luteovirus-like particles that were not observed in similar preparations from asymptomatic sugarcane cultivars. Similarly, low molecular weight dsRNAs resembling the subgenomic dsRNA species associated with other luteovirus infections have been consistently isolated from YLS-infected but not healthy sugarcane. Finally, luteovirus-specific oligonucleotide sequences have consistently primed the amplification by reverse transcriptase PCR of a 530 bp product from total nucleic acid extracts of YLS-infected but not healthy sugarcane. Thus the available evidence from electron microscopic observation, dsRNA analysis and reverse transcriptase PCR amplification suggests that one type of YLS syndrome, occurring in Brazil, Florida and Australia, is associated with infection by a luteovirus-like agent.

The YLS syndrome described in Hawaii has been reported to be soil-borne, and it was concluded from dsRNA analysis that a closterovirus might be involved in the etiology of this disorder. Samples of Hawaiian YLS-infected sugarcane did not contain the putative subgenomic luteovirus-like dsRNA associated with YLS in Florida and Brazil.

### Transmission of YLS

Mechanical inoculation of healthy sugarcane CL61-620 using crude sap or partially purified extracts from YLS-infected SP71-6163 did not result in symptom development, and neither luteovirus-like particles nor luteovirus-like dsRNAs were present in inoculated plants after six months.

To determine whether YLS was transmitted by aphids, *Melanaphis sacchari* and *Rhopalisiphum maidis* were allowed minimum 48 hour acquisition access feeds on YLS-infected SP71-6163 and CP92-1043, then transferred to healthy sugarcane cvs. CL61-620 and CP65-357, oats (*Avena sativa* Clintland 64), sweet corn (*Zea mays* cv. Early Sunglow), sorghum (*Sorghum vulgare*) and Johnson grass (*Sorghum halepense*). No symptoms developed on any test plants grown at 24–28°C, but when transferred to 18°C, aphid-inoculated plants of CP65-357 developed YLS-like symptoms and dsRNAs and a luteovirus-specific product was detected in these plants by reverse transcriptase PCR amplification. Luteovirus-like subgenomic dsRNAs were also detected in aphid-inoculated sweet corn and sugarcane CL61-620. These results indicate that the agent associated with YLS in

SP71-6163 in Brazil and in CP92-1043 in Florida is transmissible by *Melanaphis sacchari* and *Rhopalosiphum padi*, both of which colonise sugarcane.

### Detection of YLS

Both reverse transcriptase PCR amplification and dsRNA analysis have given consistent results in detecting the agent associated with YLS in Florida and Brazil, and further refinement of the PCR method would provide a sensitive and reliable detection method. Serological methods for YLS detection could also be developed.

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# Downy Mildew, Ramu Stunt and Ramu Orange Leaf

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## Abstract

Downy mildew, Ramu stunt and Ramu orange leaf are South-East Asian diseases of sugarcane, the latter two being found only in Papua New Guinea. Downy mildew, a fungal disease, is identified by foliar symptoms and down production on the underside of the leaves. It is eliminated from planting material by hot water treatment and by treatment with the fungicide metalaxyl. Ramu stunt, a viral disease can be identified by symptoms. DsRNA has also been detected in diseased material and could prove a useful diagnostic tool. No eradication studies have been reported. Ramu orange leaf has not been researched extensively. It is thought that a fungus belonging to the Exobasidiales could be the causal agent. It is a systemic disease producing characteristic leaf symptoms. Hot water treatment of planting material would probably eliminate the disease. All three diseases could prove important in the exchange of germplasm from South-East Asia, particularly as this region is a source of genetic diversity in *Saccharum* spp.

**DOWNY MILDEW** (*Peronosclerospora sacchari* [T. Miyake]) Shirai and K. Hara, Ramu stunt and Ramu orange leaf have their origin in South-East Asia, the latter two being found only in Papua New Guinea. Downy mildew has been reported in Australia, Fiji, India, Indonesia, Japan, Papua New Guinea, the Philippines, Taiwan and Thailand though it has since been eradicated from Australia.

## Downy Mildew

### Identification

The basis for downy mildew identification is leaf symptoms, particularly the characteristic leaf streaking and down (conidia) production on the underside of leaves. These symptoms are specific and quite easily separated from those of other diseases. As a systemic disease, downy mildew also causes severe growth stunting in highly susceptible cultivars.

Leaf streaks initially are pale to light yellow, generally 1–3 mm in width though much wider in some varieties. Streaks run parallel to the leaf venation and are separated by healthy green tissue (Leu and Egan 1989). Symptoms are equally evident on both leaf surfaces though sporulation is mainly confined to the lower surface. As the symptoms progress, streak colour changes from pale yellow to yellow and finally to a dark brick-red. *P. sacchari* produces two types of spores, conidia and oospores, during the cooler months. Oospore production is accompanied by leaf splitting, a phenomenon that probably would not occur under normal quarantine conditions. Most abundant production of

conidia occurs at 100% relative humidity (RH) at 22–25°C. Production is sparse below 15°C and ceases below 86% RH or above 31°C (Leu and Egan 1989). In the field, conidia are produced after 11.30 pm, peaking between 1.30 and 2.30 am and ceasing after 5.30 am.

Detection in quarantine would rely on observation of leaf streaking and confirmation by incubation of suspect leaves in a humid chamber to induce down production.

### Eradication

Downy mildew is eliminated from planting material by hot water treatment. Hughes (1954) showed that a treatment at 52°C for 30 minutes eliminated the disease. Wang (1957) treated cane at 45°C for 60 minutes followed by 52°C for 60 minutes one day later and eliminated downy mildew. Chu (1965) eliminated downy mildew with a 50°C 120 minute treatment. The specific fungicide metalaxyl (Ridomil) also eliminates the disease from planting material when setts are dipped in a 1.25 g a.i./L solution for 5 minutes (James 1983). Foliar sprays of metalaxyl have eliminated the disease from growing crops in Papua New Guinea.

## Ramu Stunt

### Identification

Ramu stunt is thought to be a viral disease with an insect vector (*Eumetopina* spp.) (Kuniata et al. 1994). Field identification of Ramu stunt is based on leaf symptoms and associated growth stunting. The symptoms of Ramu stunt are somewhat variable, grading from a yellow-green leaf mottling or striping, often more prominent on one half of the leaf blade, to a general leaf yellowing and an accompanying short, stiff

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and erect leaf habit. Symptoms are equally evident on both sides of the leaf. Such symptoms would normally be detected in quarantine if growth conditions were adequate. However, if plants were nutritionally stressed, the disease could be a little difficult to identify, particularly in tolerant varieties.

Jones (1989) reported dsRNA associated with diseased material. This finding offers an alternative method for detection in quarantine, though further research is required to determine the reliability and sensitivity of the method.

### Eradication

Infection of susceptible cultivars usually leads to plant death. No cases of spontaneous recovery from infection have been reported or of eradication with hot water treatment, though such studies have been very limited.

## Ramu Orange Leaf

### Identification

Identification of the disease is by symptoms, the most prominent of which are a fine orange striping of the leaves. Initial symptoms are a pale green band around the base of the spindle leaf. As the leaf expands, disease symptoms progress up the leaf. At the margin of the band, symptoms grade into thin (0.25–3.00 mm) streaks of variable length, which in turn merge into normal green leaf tissue. This streaking shows some similarity to downy mildew. As the leaf ages, symptoms deepen in colour from pale green, to yellow and to orange. This is more pronounced on the lower leaf surface, particularly when sporulation, which occurs only on the lower leaf surface, has taken place. The orange coloured spores are produced on erumpent hyphal masses that run parallel to the leaf venation.

No other identification tests have been developed. The causal agent, a fungus belonging to the Exobasidiaceae, has not been well characterised.

### Eradication

No eradication studies have been conducted. A hot water treatment, such as used in quarantine procedures, could prove successful. There is some doubt whether

infected sett material would germinate since the disease usually leads to stalk death in the field.

## Importance of Diseases in Germplasm Exchange

The occurrence of downy mildew, Ramu stunt and Ramu orange leaf in South-East Asia is of quarantine significance since the region is a centre of diversity of *Saccharum* spp. Downy mildew, Ramu stunt and Ramu orange leaf are all endemic in Papua New Guinea and probably widely distributed in various *Saccharum* spp. Future germplasm collection expeditions throughout the region would need to take account of these diseases.

The likelihood of their interception in quarantine is low since the diseases occur in relatively few countries. Downy mildew would become a disease of major significance if quarantine were breached. Its effect would vary by country. Resistance screening trials conducted in Papua New Guinea suggest that the susceptibility of cultivars from different breeding programs is not uniform. This is illustrated in Table 1.

If Ramu stunt passed through quarantine, its effect on crop production would depend on the presence of a suitable vector. If a vector were present, the disease could cause substantial yield losses in susceptible cultivars. Not enough is known about Ramu orange leaf to predict what its effect on yields would be.

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**Table 1.** Percentage of cultivars from different countries showing resistant and susceptible reactions to downy mildew in trials conducted in Papua New Guinea from 1984 to 1992.

Resistance rating	Percentage of cultivars tested				
	Barbados (B)	India (Co)	USA (Hawaii, H)	Australia (Q)	Australia (CSR)
Resistant (2)	53	48	47	25	50
Susceptible (8)	16	19	22	52	11
Number of cultivars tested	19	21	32	52	18

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# Ratoon Stunting Disease

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## Abstract

Ratoon stunting disease is one of the most important diseases of sugarcane. Owing to poor symptom expression and the fastidious nature of the bacterium, detection is a problem, resulting in a high risk for dissemination through exchange of germplasm. Several serological techniques have been adopted for diagnosis, and recently molecular methods have been investigated. The improved techniques should allow more efficient detection of latent infection and thereby ensure the movement of clean material.

RATOON STUNTING DISEASE, caused by *Clavibacter xyli* ssp. *xyli*, is an important bacterial disease of sugarcane. It is probably present wherever sugarcane is grown (Frison and Putter 1993). Identification of the disease is difficult because of the poor manifestation of symptoms and the fastidious nature of the bacterium. Thus there is a high risk of dissemination through germplasm exchange.

## Symptoms

External symptoms consist of stunted growth and thin stalks. They are most pronounced in susceptible clones, especially after conditions of stress such as dry spells during the growing period (Gillaspie and Teakle 1989). Ratoon crops are more severely affected and the internodal regions in mature cane are shortened, especially at the lower part of the cane. Not all stalks are affected in one stool, but their number is often reduced. Internal symptoms include salmon pink to orange discolourations as commas or bent forms in the nodal regions when cut longitudinally (Gillaspie and Teakle 1989). In young shoots a pink discolouration of the apical meristem can also be observed.

## Identification and Detection

Identification based on the presence of the causal organism is more reliable. The bacterium, *Clavibacter xyli* ssp. *xyli* was first isolated by Davis et al. (1980). Isolation on culture medium from diseased sap is slow as the bacterium takes a minimum of fifteen days to appear as microcolonies.

Phase contrast microscopy has been used for diagnosis of the bacterium directly from sap extracts, where typical straight or slightly curved rods are observed. This method is reliable for examining fresh cane stalks and, although the bacterium is systemic in the plant, it is recommended to test the lower nodal

parts of the stalk where a higher concentration of the bacterium is found.

Serological techniques have also been applied for ratoon stunting disease. Indirect enzyme-linked immunosorbent assay (ELISA) has not proved to be useful as a large number of cells must be present before a positive reaction is obtained (Gillaspie and Teakle 1989). Indirect immunofluorescent microscopy has been found to be more reliable (Harris and Gillaspie 1978; Davis and Dean 1984; Mauritius Sugar Industry Research Institute 1989). Rott et al. (1989) could detect  $2 \times 10^3$  cells/mL from infected cane juice by indirect immunofluorescent microscopy. Using an axenic culture of *Clavibacter xyli* ssp. *xyli*, the sensitivity of the test has been found to be  $1 \times 10^3$  cells/mL or 10 cells per aliquot spotted on one well of an indirect immunofluorescent microscopy slide.

Several improved serological techniques such as liquid transfer enzyme immunoassay and fluorescent antibody direct count on filters have been evaluated and were found to be very sensitive as they could detect as few as  $5 \times 10^3$  cells/mL (Leaman et al. 1991). However, these tests were found to be either difficult to perform or useful for screening large number of samples (Leaman et al. 1991).

Recently, several teams have developed molecular techniques for detecting the ratoon stunting disease bacterium. Monoclonal antibodies were produced by Martinez et al. (1991) and by Lin and Chen (1994). The latter could detect  $10^5$ – $10^6$  cells/mL in indirect ELISA with some clones. DNA probes have also been produced by Lin and Chen (1994) and these could detect 0.19–0.75 ng of homologous DNA by hybridisation and, with intact cells, a sensitivity level of  $10^4$ – $10^5$  cells/mL was reached. Random amplified polymorphic DNA (RAPD) using 10 mer oligonucleotide primers have proved to be useful in detecting as few as 100 cells/mL (Petravovits et al. 1994).

## Elimination of Ratoon Stunting Disease

Improved techniques for diagnosing *Clavibacter xyli* ssp. *xyli* will help to control the disease more

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efficiently. The dual hot water treatment consisting of a short hot water treatment followed by a three hour treatment and the cold soak – long hot water treatment are recommended for eliminating the bacterium in cuttings (Frison and Putter 1993).

### Importance in Germplasm Exchange

Ratoon stunting disease is one of the most important quarantine diseases because of its latency. Emphasis should be put on indexing mother plants from which cuttings are obtained before export, and tissue culture plantlets free of ratoon stunting disease should be envisaged for exchange of germplasm. Moreover, exports of highly susceptible clones should be discouraged.

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# Gumming Disease

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## Abstract

The latent phase of gumming disease contributes to its spread through infected setts, and therapy is unknown. Variation among isolates of the bacterium has been observed, indicating a heterogeneous population. Thus there is a danger of introducing new strains when germplasm is exchanged. Detection can be carried out by isolation, immunofluorescent microscopy using monoclonal antibodies as well as DNA probes.

GUMMING DISEASE is caused by the bacterium *Xanthomonas campestris* pv. *vasculorum* (Cobb) Dye. The description of the disease, its epidemiology, transmission and control have been reviewed by Ricaud and Autrey (1989). Gumming disease has been reported in 24 countries (Frison and Putter 1993).

## Symptoms

Gumming disease produces 4–5 mm wide yellow foliar stripes that progress toward the base of the leaf. A distinctly red foliar stripe as opposed to the yellow one has also been described (Ricaud and Autrey 1989). The systemic phase of the disease is characterised by partial or total chlorosis of new leaves, stalk deformation and knife-cut lesions, internal discolouration of nodes and internodes, gum pockets, necrosis of the apex and death of the stalk.

## Strain Variation

Isolates that show a differential host reaction to sugarcane varieties have been reported, indicating the presence of races (Antoine and Hayward 1962). Three races are present in Mauritius (Mauritius Sugar Industry Research Institute 1983). Qhobela and Claflin (1992) found two broad geographical groups by RFLP (restriction fragment length polymorphism) analysis: Southern Africa (South Africa and Zimbabwe) and Eastern Africa (Mauritius, Réunion and Australia). A comparison of isolates from various parts of the world by Dookun (1993) using fatty acid profiling, and polyclonal and monoclonal antibodies revealed that five groups are present, indicating heterogeneity in the pathogen.

## Identification and Detection

The bacterium is easily isolated from foliar stripes and nodal tissues on sucrose peptone medium where it

forms yellow and round colonies producing abundant exopolysaccharide. It is a gram negative rod-shaped bacterium with 12 main fatty acids (Dookun 1993; Stead 1989). Races can be distinguished on Keyman's tetrazolium medium (Mauritius Sugar Industry Research Institute 1983) and nutrient yeast extract glycerol agar (Dookun 1993).

The pathogen can also be diagnosed by serological tests. Polyclonal antibodies, however, cross-react with the leaf scald pathogen. Detection by monoclonal antibodies are, however, less sensitive and can detect  $1 \times 10^8$  cells/mL compared with  $1 \times 10^5$  cells/mL with polyclonal antisera in a dot blot test. With indirect enzyme-linked immunosorbent assay using monoclonals, a sensitivity of  $1 \times 10^6$  cells/mL was reached (Dookun 1993). Detection by nucleic acid spot hybridisation is also feasible. It is possible to detect the pathogen from infected plant sap using these tests.

## Elimination of the Pathogen

The dual water treatment of setts is not as effective on gumming disease as it is for other sett-borne diseases. The incidence of the disease has been reported to be reduced after heat treatment and a curative effect may occur in slightly contaminated planting material (Mauritius Sugar Industry Research Institute 1978).

## Importance in Germplasm Exchange

Gumming disease may be moved from country to country through latently infected cuttings. As no curative treatment is available, caution should be exerted when setts are chosen for export. The exchange of highly susceptible clones should be avoided. The occurrence of strains also highlights the quarantine importance of this pathogen.

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# Sugarcane Mosaic and Fiji Disease

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## Abstract

Sugarcane mosaic and Fiji disease are both important viral diseases of sugarcane. These diseases can result in substantial yield reduction in infected plants, or the reallocation of significant resources to roguing campaigns or to breeding programs for control. These diseases are caused by a potyvirus with a single-stranded RNA genome and a fivirus with a double-stranded RNA genome respectively. Identification of quarantined germplasm infected with these viruses can be difficult, especially for Fiji disease virus which, in some sugarcane clones, requires several months to develop the diagnostic galls. Sensitive and specific reverse transcriptase polymerase chain reaction tests have been developed to detect these viruses in sugarcane germplasm. A high titre antiserum to mosaic virus is also available for screening germplasm. The application of these tests to screen quarantined clones will minimise the risks associated with these viruses during the exchange of germplasm.

## Sugarcane Mosaic Virus

SUGARCANE MOSAIC POTYVIRUS (SCMV) is a member of the potyviridae, a viral group characterised by long flexuous viral particles and a single-stranded positive sense linear RNA genome approximately 10 kb in size. This virus causes mosaic, one of the most widely distributed diseases of sugarcane. Mosaic is visually identified by the chlorotic pattern on the leaf blade. Initially this pattern resembles a mosaic patchwork, but it usually progresses through to extensive chlorotic areas surrounding dark green regions in the leaf laminae. Some sugarcane clones show more intense reactions than others, including reddening or necrosis of infected tissue. Several strains of SCMV exist. These strains differ in their ability to infect and in the degree of symptoms produced.

Until recently SCMV was regarded as a single potyvirus group that consisted of a large number of strains. The current classification system has the original SCMV grouping consisting of four distinct potyviruses: SCMV, sorghum mosaic virus (SrMV), Johnson grass mosaic virus (JGMV) and maize dwarf mosaic virus (MDMV) (Shukla et al. 1992). SCMV now contains SCMV-A, -B, -D, -E and MDMV-B, while SrMV contains SCMV-H, -I, and -M (Shukla et al. 1992). For sugarcane pathologists and breeders this new classification may cause confusion as H, I and M strains all infect sugarcane.

SCMV is transmitted by infected sett material, mechanically and by a number of aphid species in different genera including the maize aphid *Rhopalosiphum maidis* Fitch.

## Identification

The symptoms of mosaic infection in sugarcane are relatively easy to distinguish, especially in the youngest unfurled leaf. Identification to strain level requires the use of a set of plant differentials. In the Australian sugarcane quarantine system, little importance has been attached to identifying the infecting strain, as confirming the presence of SCMV or destroying on suspicion is the priority. In the past few years SCMV was intercepted in germplasm from China; the diagnosis was confirmed by examining leaf dips with a transmission electron microscope and the material was destroyed. With emphasis on detecting all strains of SCMV, tests based on DNA probe and reverse transcriptase-polymerase chain reaction (RT-PCR) were developed (Smith and Van de Velde 1994). These tests were based on the findings of McKern et al. (1991) who reported that the central portion of the coat protein of SCMV strains showed strong homology at the amino acid level, although the level of homology at the nucleotide level was unknown as only the coat protein coding region of strain A had been sequenced (Frenkel et al. 1991). This test detects the SCMV strains A, B, D and E, but not SrMV strains I, H, and M. However, a high titre polyclonal antiserum raised against recombinant SCMV-A coat protein (Smith et al. 1995) does recognise all of the above strains (M.S. Irely, personal communication).

## Eradication

SCMV has been eradicated from sugarcane germplasm by serial hot water treatment of infected buds (Benda 1972), apical meristem culture and tissue culture or heat and tissue culture combinations (Leu 1972). Tissue culture of serially heat treated setts has been successfully applied to eradicate SCMV from sugarcane germplasm (Waterworth and Kahn 1978). This technique should be the most suitable to apply to

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rescue important quarantined germplasm, especially as a very sensitive RT-PCR test is available to assay the treated material. A clean source of cane and regular inspection and assay for SCMV will minimise the risks associated with quarantine of sugarcane germplasm.

### Fiji Disease Virus

Fiji disease is caused by Fiji disease fivirus (FDV), the type member of subgroup 2 of the plant reoviruses. The only definitive symptom of FDV infection of sugarcane is raised whitish galls on the abaxial side of the leaf, although stunting, side shooting and a chewed appearance of the emerging leaves are non-specific symptoms. The galls are the result of extensive cell proliferation of the vascular tissues and contain most of the virus present in the plant. FDV is one of the most important viral diseases of sugarcane. Epidemics in Fiji and Australia have seriously threatened the sugar industries in both countries. At the peak of the Fiji disease epidemic in southern Queensland, Australia, in the late 1970s over 70 million stools of cane were estimated to have been infected. The use of resistant varieties and healthy plant sources finally brought the disease under control. FDV is spread in infected sett material and by plant-hoppers in the Delphacid genus *Perkinsiella* including *P. saccharicida* Kirk, *P. vastatrix* Breddin and *P. vitiensis* Kirk.

### Identification

The presence of galls on the abaxial side of the leaf is the only definitive symptom of FDV infection. The time for gall formation can be quite variable and may even require induction by ratooning. This makes diagnosis by symptomatology difficult, especially in moderately resistant cultivars that spend only one year in quarantine. Molecular based tests (DNA probe) (Smith et al. 1994) and RT-PCR (Smith et al. 1992; Smith and Van de Velde 1994) have been developed. The RT-PCR test is sufficiently sensitive to detect FDV in the equivalent of 25 ng of tissue or approximately 30 viral particles (Smith et al. 1992). A test of this sensitivity was required as DNA probes could not routinely diagnose FDV infection prior to gall development (Smith et al. 1994). The RT-PCR test has yet to be trialled to ascertain its effectiveness for detecting FDV in asymptomatic plants.

### Eradication

FDV has been eradicated from infected germplasm by a combination of heat treatment and tissue culture of isolated axillary buds (Wagih et al. 1995). However, FDV was eradicated from only 28 per cent of the cultures as judged by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), so this method requires additional research and the

application of the more sensitive RT-PCR test before routine application. Clean sources of cane and regular inspections and testing will minimise the probability of FDV-infected germplasm being released from quarantine.

### Multiplex Reverse Transcriptase PCR

SCMV and FDV can be detected in the same sample by duplex RT-PCR (Smith and Van de Velde 1994). By adding primers specific to each pathogen to the nucleic acid extract from the plant, both viruses can be detected in the same reaction. While some work is still necessary to optimise this reaction, there are economic and logistic benefits to using duplex or multiplex RT-PCR for detecting sugarcane viruses with RNA genomes. A multiplex RT-PCR test for five different legume viruses has been developed (Bariana et al. 1993), so adding to the existing sugarcane multiplex RT-PCR test is feasible when tests are available for other RNA pathogens such as sugarcane mild mosaic virus and peanut clump virus. Similarly, a multiplex PCR test could be developed for sugarcane viruses with a DNA genome like sugarcane bacilliform and maize streak viruses.

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# Leaf Scald Disease

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## Abstract

Leaf scald is often a latent disease of sugarcane that has been inadvertently spread throughout the world with infected cuttings from symptomless plants. It is associated with symptoms that can vary from a single pencil-line leaf streak to death of the entire plant. Serological techniques are most frequently used to detect *Xanthomonas albilineans* in a host plant but selective media have been developed to aid in diagnosis. Leaf scald is a problem in germplasm exchange. Methods such as hot water treatments exist to avoid the spread from one geographical location to another.

LEAF SCALD caused by *Xanthomonas albilineans* is one of the major diseases of sugarcane (Ricaud and Ryan 1989). It was first recorded in Australia in 1911 by North, but strong presumptive evidence exists that the disease was prevalent in the Fiji Islands in 1908, if not earlier (Martin and Robinson 1961).

Sugarcane infected with leaf scald disease exhibits several symptoms: narrow white to yellow 'pencil-line' streaks on the leaf blades running parallel to the leaf veins, necrosis of the leaf tissue around these lines extending progressively from the tip toward the base of the leaf, chlorosis or whitening of the leaf tissue, wilting of the foliage with the leaf tips curling inward, development of side shoots in mature stalks that exhibit the same symptoms as the stalk foliage, and reddening of vascular bundles within the stalk.

Use of resistant varieties is the most effective means of control. However, a major factor impeding efforts to control leaf scald has been the frequent occurrence of a latency phase in infected plants (Ricaud and Ryan 1989; Rott et al. 1988a). Sugarcane can often tolerate the pathogen for several weeks or even months without exhibiting any symptoms, or the symptoms expressed are so inconspicuous that they escape detection. As a consequence, the disease has been inadvertently spread throughout the world with infected cuttings from symptomless plants. Since its discovery, leaf scald has appeared in all sugarcane producing continents, and the number of countries with this problem continues to increase. To date, it has been identified in more than 60 geographic locations in the world.

Inherent in the study and management of leaf scald is the need to detect and identify the pathogen. The frequent occurrence of latent infections has greatly limited the usefulness of diagnosis based on visual

symptoms. Furthermore, the fastidious nature of the pathogen has limited the use of isolation on culture medium as a means of diagnosis. However, a selective medium was recently developed, which will be very helpful in isolating *X. albilineans* (Davis et al. 1994a). To aid in the diagnosis of leaf scald, several different serological techniques, such as immunofluorescence, enzyme-linked immunosorbent assay and agglutination tests, have been developed (Autrey et al. 1989; Comstock and Irey 1992; Rott et al. 1988b). These techniques are very useful for rapid detection or identification of the pathogen but the serological variability of *X. albilineans* must be taken into consideration. Indeed, three serovars were identified after investigation of the serological characteristics of 271 strains of *X. albilineans* from 33 locations throughout the world (Rott et al. 1994). Molecular diagnostic techniques using the polymerase chain reaction to detect the pathogen are presently being investigated (Lopes and Damann 1994; Davis et al. 1995).

Leaf scald has been identified in numerous locations but it has been shown recently that different genetic groups exist (Davis et al. 1994b). Most locations are affected by only one or two genetic variants of the pathogen. The limited geographic distribution of genetic variants supports the need for programs to prevent the spread of the pathogen from one country to another. A hot water treatment can be applied to eliminate the pathogen from infected cuttings (Steindl 1971). It consists of soaking sugarcane cuttings in water at ambient temperature for 24–48 hours, followed by another soaking at 50°C for three hours. This procedure is routinely used for sugarcane quarantine at CIRAD in Montpellier.

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# Red Leaf Mottle

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## Abstract

Red leaf mottle of sugarcane is associated with several symptoms that can vary from wine-red spots to white stripes and chlorotic bands. The disease is caused by the peanut clump virus (sugarcane isolate). Serological tests (enzyme-linked immunosorbent assay) or electron microscopy can be used to detect the pathogen in leaves, stalks or roots. The pathogen can be easily transmitted by cuttings, and no treatment is presently known to eliminate the virus in infected material. The disease apparently occurs in only a few countries so far; therefore, great attention should be paid during germplasm exchange involving infected locations.

RED LEAF MOTTLE of sugarcane is caused by a virus similar to the peanut clump virus (PCV) (Baudin and Chatenet 1988). PCV is a furovirus that has been reported on peanut for many years but was found only recently in sugarcane. Sugarcane affected with red leaf mottle exhibits several leaf symptoms that vary according to the cultivar.

- Chlorotic stripes with yellow or rust mottling extend along the leaves; stripe width is several millimetres and may extend across the leaf blade. The entire leaf may turn brownish red with age (cvs. Co1001 and B70574).
- A large number of wine-red spots lacking a clear outline appear on both sides of the leaf blade (cv. Ragnar).
- White streaks lacking a clear border appear on the leaves of certain cultivars. Large white patches may appear in a herring-bone pattern (cvs. B51129, B51410 and B7134).

Red leaf mottle can be diagnosed by electron microscopy (leaf dip). The virus forms rigid rods of

variable length in sugarcane leaf preparations. Particle length ranged from 200 nm to 250–300 nm and the diameter was 20 nm. The PCV sugarcane isolate is highly antigenic. Antisera prepared against purified virus were used to detect the pathogen by direct double sandwich ELISA in leaves, stalks and roots of sugarcane (Baudin and Chatenet 1988).

Red leaf mottle is a soil-borne disease, but it can be easily transmitted by infected cuttings. No efficient method has yet been reported to eliminate the virus from infected cuttings. Soaking cuttings in water for three hours at 50°C did not eradicate the disease (Baudin et al. 1994). The disease has been reported in only a few countries in Africa. Therefore great attention should be paid during germplasm exchange involving affected locations.

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# Smut, Streak Virus and New Reovirus

R.A. Bailey\*

## Abstract

Smut occurs in many sugar exporting countries. The pathogen can be transmitted in setts from which it can be eliminated using hot water treatment. Sugarcane streak virus, which has been reported mainly in Africa, is readily detected by enzyme-linked immunosorbent assay and immunosorbent electron microscopy. The risk of the virus being transmitted in setts exported from southern Africa is regarded as small. The reovirus only recently observed is diagnosed by electron microscopy and dsRNA analysis.

## Smut

SMUT (*Ustilago scitaminea*) occurs in many countries from which sugarcane varieties are exported, the main exception being Australia. The pathogen can be transmitted in setts. Standard procedures for preparing setts for export include choosing only plants without symptoms of systemic diseases. However, if setts for export are prepared from field grown cane in areas where smut occurs, the selection of symptomless plants is no guarantee of freedom from infection, since smut may still be present.

In South Africa, setts of certain advanced prerelease genotypes are available from only the Pongola selection site in northern Natal, where smut is common. Advanced selections at this site all have some resistance to smut, but not necessarily high resistance.

Fortunately, smut is readily eliminated from setts by treatment in water at 50°C for periods of 30 minutes or longer. Since recommended quarantine procedures include the hot water treatment of setts either by the exporter or on receipt by the importer, or both, and for two hours or longer on one or two occasions before varieties are released from closed quarantine, smut should pose no quarantine risk.

Mycelium of *U. scitaminea* can be observed in microscopic slide preparations of infected stalk tissue, but this is not a fail-safe detection technique.

## Sugarcane Streak Virus

Streak virus has been reported in many African sugarcane producing countries and also in Réunion, Mauritius, India and Pakistan. In South Africa, streak was common, almost ubiquitous, in the dominant variety Uba in the 1930s but virtually disappeared with the demise of that variety; it has not been seen in

commercial cane for more than 20 years. There appear to have been no records of streak in sugarcane from other countries in southern and central Africa for a similar period, although the maize strain of the virus is common in maize.

Advanced genotypes undergoing selection in South Africa are still tested for resistance in natural exposure trials using infected Uba as spreader plants. Very few genotypes (less than 1%) develop symptoms. This indicates that the parent varieties being used have a high degree of resistance.

Although streak virus cannot be eliminated from setts by conventional means, the risk of the virus being transmitted in setts exported from southern Africa is regarded as small because of the rarity of the disease in sugarcane.

Streak virus can be readily detected by enzyme-linked immunosorbent assay (ELISA) and immunosorbent electron microscopy (ISEM) using antisera prepared from the maize or sugarcane strains of the pathogen. ISEM provides an accurate diagnosis because of the distinctive appearance of the particles of this geminivirus. A specific antiserum prepared from infected sugarcane is available from SASEX.

## New Reovirus

This new disease was first observed in a prerelease genotype in South Africa in January 1994. It has since been found on a number of released varieties and new genotypes on farms and selection sites in various localities. Symptoms have been limited to small galls occurring in chains on lower leaf surfaces and on leaf sheaths. The galls are often inconspicuous and sparse.

No effects on plant growth have been observed. Limited studies have indicated that the disease is not transmitted in setts.

A spherical virus particle similar in size to the Fiji disease virus has consistently been observed by electron microscopy in preparations from galls but not from other tissues. Analysis has shown the particle to be a dsRNA virus. Three DNA fragments were

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prepared by amplification using primers for the Fiji disease virus; the banding patterns showed similarities to but were distinct from patterns for the Fiji disease virus. The size of the particles and other similarities to

the Fiji disease virus indicate that the virus belongs to the reovirus group.

The virus is diagnosed by electron microscopy and dsRNA analysis.

# **Workshop Summaries**

# Disease Risk Analysis and Strategic Planning for Conservation and Exchange of Sugarcane Germplasm

P.J.L. Whittle and P.H. Twine\*

## Abstract

Following the presentation of the formal papers, two workshop sessions were held. These were intended to develop consensus on:

- prioritisation of diseases in terms of the risks they pose to germplasm conservation and exchange; and
- strategic approaches required for dealing with these risks in order to adequately conserve and exchange germplasm.

This paper summarises discussions in the workshops.

## Workshop 1:

### What diseases pose the greatest risk to germplasm conservation and exchange?

Individually, and then in eight groups of four or five people, delegates rated 29 diseases according to their economic significance and their manageability. The group ratings were tabulated, and the two attributes were plotted against each other to give an index of risk (Figure 1).

This established four broad disease clusters:

- six diseases rated highly for potential economic significance and difficulty of management;
- four diseases of lower economic risk that pose management difficulties;
- six diseases of high economic risk that can be managed easily in quarantine; and
- thirteen diseases that in general do not present significant concerns.

These ratings were generally agreed on, although there were some differences in interpretation of the two attributes.

*Economic significance* was interpreted in terms of present losses and also potential losses. For example, gumming poses a greater economic risk in quarantine to Australia, where it has not occurred for 40 years, than it does to Mauritius, where it is endemic. Also, estimation of potential economic significance needs to include consideration of risks to valued alternative host species.

*Manageability* was intended to mean the ease of excluding, eradicating or detecting a pathogen in quarantine, or selecting an alternative clean source. Another interpretation that led to some discussion was the ease of managing the disease after ingress to an industry.

There was limited opportunity for detailed debate on each disease and its ratings. However, from the formal papers, a few summary points that clarify threats from the diseases can be made. (For further details, refer to Frison, E.A. and Putter, C.A.J., eds, *Technical Guidelines for the Safe Movement of Sugarcane Germplasm*, Food and Agriculture Organisation of the United Nations, Rome, and International Board for Plant Genetic Resources, Rome, 1993, 44 pp.)

## Diseases of high to moderate quarantine risk

- Fiji disease — very serious economically; indexing unreliable; no routine therapy; vector widespread with high risk of establishment in a new area.
- Yellow leaf syndrome — may be serious economically; no diagnostic test; pathogen not identified; no known therapy; high risk of establishment in a new area.
- Mosaic — serious economically; indexing reliable; therapy feasible but complex; widely distributed, but risk of strain establishment in a new area; risk to other host crops.
- Ramu stunt — very serious economically; excluded easily through its limited distribution (Papua New Guinea only); detection relies on symptoms as pathogen not identified; vector distribution limited.
- Gumming — serious economically; detection not fully reliable; no routine therapy; high risk of establishment.
- Sugarcane bacilliform virus — seriousness not established, although strains aggressive on banana have been reported; detection unreliable; no routine therapy; low risk of establishment as endemic in all areas.
- Red leaf mottle — seriousness not established; detection moderately difficult; no routine therapy; vector distribution not defined.
- Leaf scald — serious economically; detection moderately difficult; controlled by hot water treatment but may escape treatment; variant types exist.

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## Diseases of lower quarantine risk

- Smut, ratoon stunting disease, downy mildew, red rot, rust and chlorotic streak — high to moderate economic significance, but routine therapy available.
- White leaf disease/grassy shoot disease, streak and sugarcane mild mosaic virus — moderate to low economic significance; management may be difficult.
- Red stripe/top rot, mottle stripe, Fusarium, Sclerophthora, bacterial mottle, wilt, pineapple disease, striate mosaic, spike, sembur, dwarf and leaf scorch — low economic significance; high manageability.

## Diseases not considered

- Ramu streak, Ramu scorch and Ramu orange leaf.

## Unknown diseases

Limited generic tests exist for routine indexing of unknown potential pathogens.

## Conclusions

High quarantine risks in terms of both potential economic significance and difficulty of management in quarantine are posed by only a small group of diseases, providing minimum post-entry quarantine practices include phytosanitary sourcing, long hot water therapy, and observation of plant and ratoon growth in quarantine. Ramu stunt can arguably be downgraded because of the restricted range of itself and its vector. Red leaf

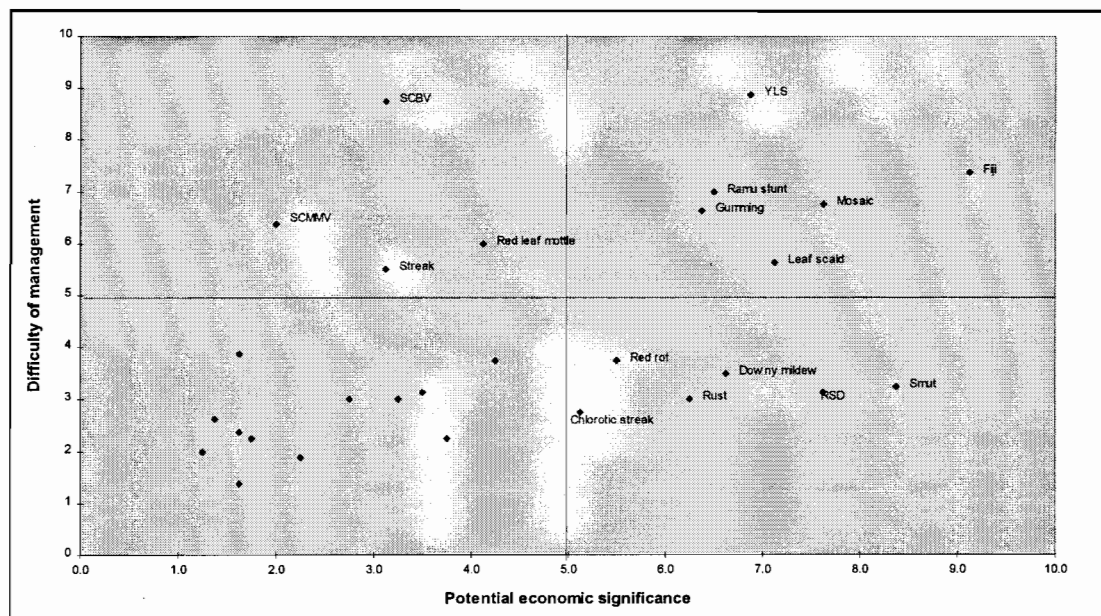
mottle, sugarcane mild mosaic virus and streak, while not rated highly in economic potential, pose concern because of difficulties in detection and management in quarantine. Several countries place high importance on sugarcane bacilliform virus in germplasm exchange, although its economic seriousness is not clear. Its significance for bananas must be taken into account in analysing its pest risk status.

The discussions demonstrated that continued international strategic planning and research collaboration is required to address numerous areas of weakness in disease risk management.

## Workshop 2:

### Planning ongoing international access to sugarcane germplasm

The workshop session opened with short commentaries from four speakers: Dr E. Henzell, Dr C. Ryan, Dr N. Berding and Mr B. Croft. Respectively, they provided perspectives from a research funder, a research provider, a plant breeder and a plant pathologist. Delegates representing each country and/or organisation (Copersucar, RSC, CIRAD, BSES, SBI-ICAR, MSIRI, ISRI, SRA, USA, SASEX) then commented individually on these leading remarks. Later, delegates were divided into several groups (Africa, plant breeders, developing countries, quarantine specialists, and virologists). Each



**Figure 1.** Plot of potential economic significance and manageability in quarantine of 29 sugarcane diseases. Economic significance is rated 0–10 for increasing significance. Manageability is rated 0–10 for increasing difficulty. Data are means of ratings by eight groups of four to five people. Diseases in lower left quadrant are grassy shoot/white leaf, leaf scorch, wilt, pineapple disease, red stripe/top rot, striate mosaic, Fusarium, Sclerophthora, bacterial mottle, dwarf, spike, sembur and mottle stripe.

of these groups then reported back. Further group discussions were held on key topics. Finally, Dr Henzell provided concluding remarks.

### Disease risks

Each leading speaker commented that delegates' perceptions of risks posed by each disease seemed to be in close agreement on most points. Some delegates' positions differed due to national or industry situations. However, it was possible to identify common objectives that could be dealt with by international agreements and initiatives in germplasm exchange protocols.

### Safety of exchange

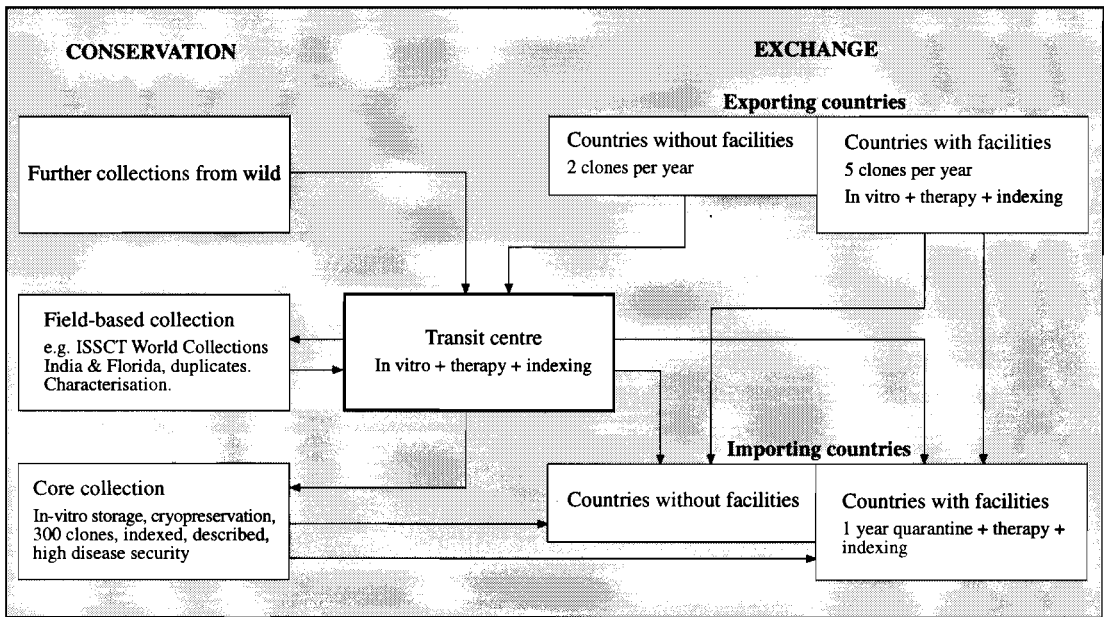
One common view was that there are major concerns about safety of germplasm exchange. These concerns and uncertainty have led to import moratoriums in several countries. Several new diseases have emerged for which indexing tests are not available. For example, yellow leaf syndrome has evidently passed through quarantine and become established in Australia and South Africa. Research is urgently needed to identify the yellow leaf syndrome pathogen, develop an indexing test for it, and determine its economic importance and biology. The importance of the sugarcane bacilliform virus needs clarification to resolve uncertainty and to enable safe exchange. A range of generic tests is also needed for unidentified potential pathogens. For some well-known pathogens, more robust and simpler tests are needed.

### Post-entry quarantine

Post-entry quarantine approaches differ between nations. The FAO/IBPGR Technical Guidelines have not been adopted in full by any country. This is possibly because national priorities and risk assessments differ and because of the prohibitive cost of applying all of the indexing tests in all places. The guidelines are likely to be implemented only in a collaborative system, where laboratories complement each other in physical and technical capacity. Collaboration is also required in addressing gaps in the Technical Guidelines. The formal papers indicated current research areas and the gaps will now be more clearly defined. In the near future it would be beneficial for a workshop to plan collaboration on critical research areas in quarantine, including indexing and elimination of pathogens. These matters could be addressed regularly in a quarantine session of ISSCT pathology workshops.

### Collaborative exchange systems

Some collaborative post-entry systems are operating (for example, CIRAD quarantine for francophone countries, SASEX coordination of quarantine in southern Africa, and contract quarantine by BSES for RSC). There is the potential for further development of such systems on a regional or global basis. Delegates gave strong support to a model proposed by Barry Croft (Figure 2) for a global sugarcane genetic resources network (SGRN) similar to INIBAP. A 'transit centre'



**Figure 2.** A model for an international network for conservation and safe exchange of sugarcane germplasm (B. Croft, BSES). Therapy and indexing in all centres are done to agreed protocols, which are reviewed periodically.

would be responsible for indexing and therapy for the full range of tests required. Some of this work could be done at other laboratories on clones provided by the centre, as is the practice in INIBAP. As a preliminary step towards establishing an SGRN, more regional efforts could be made, such as an Asia-Pacific network.

### **Quality assurance in exchange**

Many post-entry quarantine problems can be avoided by exercising quality assurance measures in sourcing the cane. The Technical Guidelines recommend sourcing from a nursery, although a further step could be to maintain an export collection, fully indexed, in a quarantine standard glasshouse. The use of explicit quality assurance measures by suppliers would substantially reduce risk to importers, allowing savings in cost, rigour and duration of post-entry quarantine. Several delegates saw value in a proposal to develop memorandums of understanding to conduct germplasm exchange under agreed standards. Less well-resourced industries that cannot participate would be relying on more inclusive strategies (such as a global SGRN), which would be developed in the longer term.

### **International communications**

Implicit in these proposals is that international communication is important. The systems proposed would facilitate communications, at least between participants. ISSCT has provided a good structure for information exchange, but this structure needs to be used actively and could be built on. A directory of sugarcane pathologists and breeders would be a useful publication. There is also a need for rapid information exchange, such as could be provided by a sugarcane diseases bulletin board on the Internet. This could be used to publicise quarantine interceptions, disease outbreaks and research findings, as well as programmed periodic information such as annual quarantine clearance reports. In using these open communication methods, it is necessary to bear in mind industry or national sensitivities; it was considered unreasonable to publicise a disease finding unless a positive identification was made and the originating country was consulted. Some workers do not have access to the Internet and would require a newsletter based on bulletin board messages. This responsibility would need to be met by a volunteer or ideally by an agency sponsored by CGIAR.

### **Training**

An SGRN would provide excellent opportunities for training workers in quarantine practice, particularly for countries that have few quarantine resources. There are needs for training at levels ranging from short term vocational to postgraduate. Linkage of an SGRN to a training institution or university would therefore be advisable.

## **Germplasm conservation**

### **Content of collections**

Collections should contain accessions of specific value and should also maintain genetic diversity for long term needs that may not be anticipated. A rational approach to the content of collections would be enabled by statements from plant breeders on their anticipated requirements. The content of present collections is extensive, although several delegates noted their intentions to collect further in certain areas and genera. Genetic diversity in the wild is threatened by rising population density placing pressure on habitats and by cultural evolution away from the use of chewing canes. It is important to undertake collections soon in threatened areas.

Clear knowledge of what presently exists in collections is valuable. Efforts by India to characterise and catalogue accessions in the World Collection will greatly increase the usefulness of the collection. Molecular markers may have increasing value in clone characterisation. It may be possible to designate a 'core collection' (Figure 2) consisting of, say, 300 clones that have short term priority for access by breeders. These could be maintained separately under a high degree of quarantine security involving thorough indexing, as well as general phenotypic characterisation.

### **State of World Collections in India and the USA**

Both ISSCT World Collections are under threat from incursion by diseases that cannot presently be eradicated. The global SGRN proposed would assist by improving collaboration on eradication and indexing. It may also help to resolve funding issues that threaten the collections through a lack of resources. Neither of the World Collections has received financial support from other countries, and both are in need of support. ISSCT has addressed this issue in the past, but could not gain commitments from its members. New efforts are required.

Collections need to be duplicated to allow replacement in the event of losses or infection by systemic and non-eradicable disease. The Florida collection is presently being rebuilt, after hurricane damage, from duplicates that were sent previously to Brazil. At the site of the proposed core collections, it may be possible to use advanced technology (for example, cryopreservation) for mass storage of a duplicate set of the main collection or of a larger collection. India is commencing a program of tissue culture storage of clones in its collection, but this has limited funding. Seed storage could be used as a supplementary means conserving genes as opposed to genotypes.

### **Access to collections**

Many delegates expressed demand for germplasm in breeding programs. Exchange is undertaken willingly by most countries for demonstrable mutual benefits. The varieties obtained in exchange must undergo a

selection process lasting 10 years or more before they may be deployed commercially, so the donor country is unlikely to lose a commercial advantage by exchanging its variety. There have been relatively few requests for basic germplasm from the World Collections. Demand is expected to increase when collections are better characterised phenotypically and at the molecular level. Accessibility is greatly improved by adequate characterisation and cataloguing, and further efforts are required in this regard. Export from the collections needs to be done with reasonable quality assurance standards.

#### **Establishment of a sugarcane genetic resources network**

Genetic resources networks such as INIBAP are established under the umbrella of CGIAR. Discussions can be initiated on prospects for CGIAR fostering an SGRN. IPGRI's support is imperative. The broad consensus of the workshop provides argument for seeking such support. Backing from governments of sugarcane growing countries is vital. Other funding organisations such as national aid bodies may assist with funding in initial stages. National funding organisations may also fund network-based activities if national priorities are addressed. As an SGRN began to operate, funding would be required from a diversity of sources, including users. Regional networks such as an African or Asia-Pacific network could be established with local aid funding and could later be integrated into an SGRN.

## **Conclusions**

This international meeting was convened because of widespread concerns about conservation and safe exchange of sugarcane germplasm. The workshops identified that increased international cooperation was a key requirement for the solution of these problems. Proposals were initiated at the workshop for developing international structures that would address the concerns that led to the workshop in the areas of:

- germplasm exchange
  - genetic resources exchange scheme
  - indexing facility for diseases
  - guidelines for safe exchange
  - quarantine protocols developed
- germplasm conservation
  - world collection (in situ, in vitro, seed)
  - characterisation
- Information exchange
  - networks
  - training
  - research and development promotion.

The development of a sugar germplasm initiative under IPGRI (like the INIBAP and COGENT networks) was discussed. This would need special support, perhaps from groups such as the World Bank (Common Commodities Fund), ACIAR and SRDC. The meeting organisers, developing country representatives, and Dr E. Henzell representing TAC, all agreed to pursue the initiative through CGIAR and other appropriate international groups.