FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS

INTERNATIONAL BOARD FOR
PLANT GENETIC RESOURCES

FAO/IBPGR TECHNICAL GUIDELINES
FOR THE
SAFE MOVEMENT OF EDIBLE AROID GERMPLASM

Edited by
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In collaboration with

RESEARCH INSTITUTE FOR PLANT PROTECTION

INTRODUCTION

Collecting, conservation and utilization of plant genetic resources and their global distribution are essential components of international crop improvement programmes.

Inevitably, the movement of germplasm involves a risk of accidentally introducing plant quarantine pests along with the host plant material; in particular, cryptic pathogens such as viruses pose a special risk. In order to minimize this risk, effective testing (indexing) procedures are required to ensure that distributed material is free of pests that are of quarantine concern.

The ever increasing volume of germplasm exchanged internationally, coupled with recent rapid advances in biotechnology, has created a pressing need for crop-specific overviews of the existing knowledge in all disciplines relating to the phytosanitary safety of germplasm transfer. This has prompted FAO and IBPGR to launch a collaborative programme for the safe and expeditious movement of germplasm reflecting the complementarity of their mandates with regard to the safe movement of germplasm. FAO has a long-standing mandate to assist its member governments to strengthen their Plant Quarantine Services, while IBPGR's mandate - *inter alia* - is to further the collecting, conservation and use of the genetic diversity of useful plants for the benefit of people throughout the world.

The aim of the joint FAO/IBPGR programme is to generate a series of crop-specific technical guidelines that provide relevant information on disease indexing and other procedures that will help to ensure phytosanitary safety when germplasm is moved internationally.

The technical guidelines are produced by meetings of panels of experts on the crop concerned, who have been selected in consultation with the relevant specialized institutions and research centres. The experts contribute to the elaboration of the guidelines in their private capacities and do not represent the organizations to whom they belong. FAO, IBPGR and the contributing experts cannot be held responsible for any failures resulting from the application of the present guidelines. By their nature they reflect the consensus of the crop specialists who attended the meeting, based on the best scientific knowledge available at the time of the meeting.

The technical guidelines are written in a short, direct, sometimes 'telegraphic' style, in order to keep the volume of the document to a minimum and to facilitate

updating. The guidelines are divided into two parts: The first part makes general recommendations on how best to move germplasm of the crop concerned and mentions available in termediate quarantine facilities when relevant. The second part covers the important pests and diseases of quarantine concern. The information given on a particular pest or disease does not pretend to be exhaustive but concentrates on those aspects that are most relevant to quarantine. In general, references are only given on the geographical distribution of the diseases and pests.

The present guidelines were developed at a meeting held in Wageningen, the Netherlands from 14 to 18 November 1988. The meeting was hosted by the Research Institute for Plant Protection and sponsored by the Directorate General for International Cooperation (DGIS) of the Netherlands Ministry for Development Cooperation.

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GENERAL RECOMMENDATIONS

The guidelines set out below should be followed when transferring aroid germplasm:

Seed

- Seed of *Colocasia, Xanthosoma* and other aroids pose a minimal risk of introducing exotic pathogens to new countries. If it is not essential to move particular genotypes, and if they are available, then seeds should be preferred for the movement of aroid germplasm.

- Unblemished seeds should be selected from plants which appear healthy. Seeds should be treated as described by Strauss, Michaud and Arditti (1979) and by Volin and Zettler (1976).

- On arrival in the recipient country, the seed should be germinated and the seedlings grown in post-entry quarantine for one crop cycle.

Vegetative propagating material

- Germplasm in vegetative form should be transferred as sterile, pathogen-tested plantlets growing on tissue culture medium (Arditti and Strauss, 1979; Hartman, 1974; Jackson, Ball and Arditti, 1977; Strauss and Arditti, 1980).

- Meristem-tips should be cultured either in the country of origin or at an intermediate quarantine centre. Each culture should be derived from a meristem-tip consisting of not more than two leaf primordia and not exceeding a size of 0.5 mm.

- Each culture should be indexed for microbial contamination using the protocol described by Knauss (1976) and Taylor and Knauss (1978).

- For the movement of *in vitro* cultures, neither antibiotics nor charcoal should be added to the medium.

- Each meristem accession should be given a code number for future reference.

- Plantlets should be tested for viruses in the country of origin, in an intermediate quarantine station or in post-entry quarantine. This should be carried out in a location with a tissue culture facility that has the capacity to test *in vitro* cultures for microbial contamination within plant tissues and to carry out virus-testing using bioassays (glasshouse facilities capable of growing aroids under good growing conditions) and serological tests. Electron microscopy facilities should also be accessible.

- Plantlets should be grown-out under glasshouse post-entry quarantine conditions upon receipt, for a period equivalent to one crop cycle.

- If it is essential to import corms or tops, they should be washed free of soil and fumigated, dipped in insecticide (e.g. carbaryl/malathion, white oil mixture) and treated with fungicide (e.g. captan). No plant material containing any soil or root debris should be transported abroad for any reason. The material should be subjected to strict post-entry quarantine for at least one crop cycle and should be tested for viruses.

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Intermediate quarantine stations available for edible aroids*

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PESTS OF QUARANTINE IMPORTANCE

Viral diseases

1. Alomae

Cause
A mixed infection by bobone rhabdovirus and the unnamed small bacilliform virus in certain taro types (referred to as 'male' taros in Papua New Guinea and Solomon Islands).

Symptoms
In 'male' taros, initial symptoms resemble those induced by bobone. Leaves are short and the tissues are thickened and severely distorted but remain green. Subsequently formed leaves are yellow and show varying degrees of vein clearing and distortion. Leaf production ultimately ceases and plants rot and die. Sucker symptoms resemble those of the main stalk (Fig. 1).

Geographical distribution
Papua New Guinea and Solomon Islands (Gollifer *et al.*, 1977; Jackson, 1980).

Fig. 1. Symptoms of lethal alomae disease on *Colocasia esculenta.* Older leaves have collapsed leaving younger, light green leaves, rolled and starting to decay. (Dr. G.V.H. Jackson, South Pacific Commission, Nouméa)

Transmission
The bobone virus is transmitted by the taro planthopper *(Tarophagus proserpina)* whereas the smaller bacilliform virus is transmitted by the mealybug, *Planococcus* sp.

Particle morphology
Virions of bobone are bacilliform in outline (50 x 330 nm) as are those of the smaller virus (30 x 125 nm).

Therapy
Plants free of alomae can readily be obtained by meristem-tip culture when small (0.5 mm or less) meristem tips are used. (See references p. 7.)

Indexing
Negatively stained leaf extracts examined by electron microscopy will reveal bacilliform viruses of two size ranges. A post-entry quarantine period for one crop cycle is essential to assure that the 'male' taro plants are free of the disease.

2. Bobone virus

(rhabdovirus group).

Symptoms
Severe stunting of recently planted stock, Leaves are puckered, distorted, brittle and thickened (Fig. 2). Plant eventually produces leaves with few or no symptoms. Original leaves with symptoms remain green.

Geographical distribution
Papua New Guinea, Solomon Islands (Gollifer *et al.*, 1977; Jackson, 1980). There is evidence for a mild strain of bobone in several Pacific island countries, causing vein clearing without severe leaf distortion.

Transmission
Transmission is by the delphacid planthopper *Tarophagus proserpina,* in a persistent manner.

Particle morphology
Bacilliform, 50 x 330 nm.

Alternative hosts
Philodendron selloum proved susceptible when inoculated in laboratory studies.

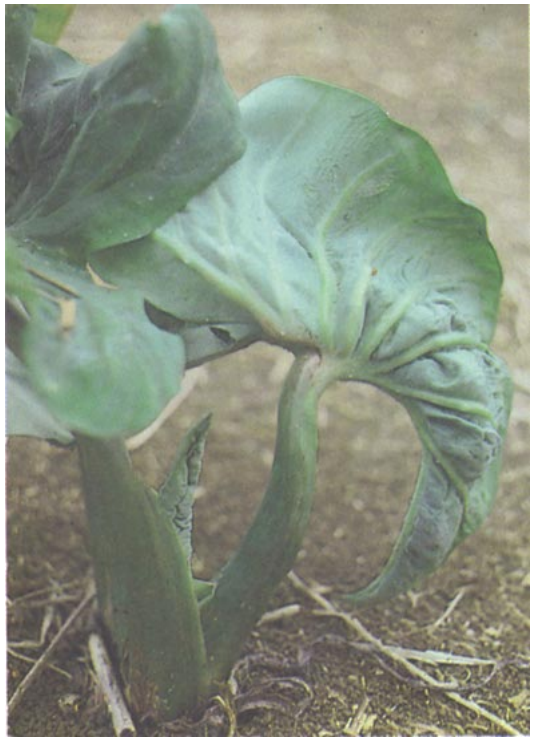

Fig. 2. Symptoms caused by the bobone rhabdovirus on *Colocasia esculenta.* (Dr. F.W. Zettler, University of Florida, Gainesville)

Therapy

Plants free of this virus can easily be obtained by meristem-tip culture when small (0.5 mm or less) meristem tips are taken. (See references p. 7.)

Indexing

Examination of negatively stained leaf extracts by electron microscopy may reveal bacilliform virus particles. Detection of diagnostic symptoms of actively growing, recently planted material is the only other reliable indexing method for this virus.

3. Dasheen mosaic virus (DMV)

(potyvirus group)

Symptoms

DMV is not lethal; its chief effect is to retard plant growth and reduce corm yield. Corms are without symptoms, but conspicuous foliar mosaic, mottle and 'feathering' symptoms are apparent. Foliar symptoms are intermittently expressed. Severity and persistence of expressed symptoms vary according to plant genotype (Figs. 3 and 4).

Fig. 3. Symptoms of dasheen mosaic virus on *Colocasia esculenta.* (Dr. F.W. Zettler, University of Florida, Gainesville)

Fig. 4. Distortion of leaves of *Colocasia esculenta* caused by a severe strain of dasheen mosaic virus. (Dr. G.V.H. Jackson, South Pacific Commission, Nouméa)

Geographical distribution
Worldwide (Jackson, 1980; Jackson, 1982; Shaw, Plumb and Jackson, 1979; Zettler and Hartman, 1986; Zettler and Hartman, 1987; Zettler and Jackson, 1979; Zettler, Abo El-Nil and Hartman, 1978). There is evidence for the occurrence of a severe strain of the virus in French Polynesia (Jackson, 1982).

Transmission
By aphids, non-persistently, under natural conditions. The aphid, *Pentalonia negronervosa*, however, is apparently not a vector.

Particle morphology
Flexuous rods, about 11 x 750 nm. Induces cylindrical inclusions, as do other potyviruses.

Alternative hosts
Alocasia, Amorphophallus, Caladium, Cyrtosperma, Dieffenbachia, Zantedeschia and other members of the Araceae.

Therapy
Attempts to eliminate this virus from infected plants through heat treatments have failed. Plants free of this virus can easily be obtained through meristem-tip culture when small (0.5 mm or less) meristem-tips are used. (See references p. 7.)

Indexing
The material should be grown for at least one crop cycle and observed for symptoms. To confirm the absence of the pathogens, one or more of the following confirmatory techniques should be used.

- *Philodendron selloum* seedlings can be used as indicator plants for mechanical inoculations. Diagnostic symptoms appear 2-3 weeks after inoculation, provided seedlings are actively growing.
- Antiserum has been prepared in Florida and the Netherlands. The sera can be used in double radial immunodiffusion tests, immunosorbent electron microscopy tests, and in direct and indirect ELISA tests.
- Electron microscopy: Flexuous rod virus par titles and/or cylindrical inclusions seen in negatively stained leaf extracts are indicative of dasheen mosaic virus, although the results should be confirmed by other, more specific methods.
- Light microscopy: Epidermal leaf strips stained in calcomine orange/Luxol brilliant green will reveal granular inclusions in the cytoplasm of dasheen mosaic virus-infected plants.

4. Small bacilliform virus

(Assigned to no virus group, but may be similar to that which causes swollen shoot of cacao.)

Symptoms

Vein clearing, especially along leaf margins. Leaf blade curved downward along margins, not thickened or distorted as in bobone (Fig. 5). Infected plants show symptoms shortly after planting, but then produce leaves without apparent symptoms.

Geographical distribution

Cook Islands, Papua New Guinea, Samoa and Solomon Islands (Gollifer *et al.*, 1977; Jackson, 1980).

Transmission

By the mealybug, *Planococcus sp.*

Particle morphology

Bacilliform, 30 x 125 nm.

Alternative hosts

All edible aroids, except *Cyrtosperma chamissonis.*

Fig. 5. Vein clearing, puckering and mosaic on leaves of *Colocasia esculenta* infected by the small bacilliform virus of the alomae complex. (Dr. G.V.H. Jackson, South Pacific Commission, Nouméa)

Therapy

Plants free of this virus can easily be obtained through meristem-tip culture when small (0.5 mm or less) meristem tips are taken.

Indexing

Examination of negatively stained leaf extracts by electron microscopy may reveal bacilliform virus particles. Otherwise, detection of diagnostic symptoms in recently planted, actively growing plants is the only reliable means thus far available for detection.

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Fungal diseases

1. Taro leaf blight

Cause
Phytophthora colocasiae

Symptoms
Leaves are attacked and the small circular, water-soaked lesions, which are the first sign of infection, rapidly enlarge, become irregular in shape, zoned, dark brown with a yellow margin and contain yellow to red droplets, which later become hard (Fig. 6). The lesions give rise to secondary colonies which cause the leaves to collapse in 10-20 days so that plants have three or four leaves while the usual number on healthy plants is six or seven. Petioles are not affected, but the spores, washed from the leaves to the soil, invade the corms after harvest through areas exposed when the suckers are removed. Losses in corm yield are 30-50% and corm rot is a serious problem (Jackson, 1977; Jackson, 1980; Holliday, 1980).

Geographical distribution
Africa, Ethiopia and Equatorial Guinea (Macias Nguema island); throughout Asia, including Indonesia, Japan, Malaysia, the Philippines and Taiwan; West Indies (Do-

Fig.6. Symptoms of *Phytophthora colocasiae* in *Colocasia escuelenta* (Dr. G.V.H. Jackson, South Pacific Commission, Nouméa)

minican Republic); and the following Pacific Islands: Guam, Hawaii, Federated States of Micronesia, Palau, Papua New Guinea, Solomon Islands. '(Anonymous, 1970; Gollifer, Jackson and Newhook, 1980).

Biology

Spores formed on the leaf lesions are spread in wind-driven rain and dew. Temperatures of 25-28°C and relative humidities of 65% during the day and 20-22°C and 100% at night with light rains or heavy dew in the morning favour the disease. Spores can survive on the planting 'tops' for up to 3 weeks after harvest and infection of the petioles sometimes occurs where these have been trimmed for planting. Infected corms could spread the disease (Gollifer, Jackson and Newhook, 1980; Holliday, 1980).

Alternative hosts

Possibly *Alocasia macrorrhiza* (Gollifer, Jackson and Newhook, 1980).

Quarantine measures

Strict quarantine measures should be observed to prevent further spread of this disease and movement of taro between countries should be limited to sterile, pathogen-tested plantlets growing in tissue culture medium.

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Insect pests

1. Taro beetles, *Papuana spp.*

Damage
Adult beetles tunnel into the corms to feed. Attack at planting can kill the planting sett, but this is uncommon and most damage occurs later when the corm develops (Figs. 7 and 8). High infestations can totally destroy the corm and even low infestations reduce marketability.

Geographical distribution
There are many species which are not widely distributed. They occur in Fiji, Kiribati, Indonesia (Irian Jaya and the Moluccas Islands), Papua New Guinea, Solomon Islands and Vanuatu (Anonymous, 1978; Jackson and Firman, 1984; MacFarlane, 1988).

Biology
The adult beetle is black and shiny, 15-20 mm long, with horns on the head which vary in size and number according to the species. Those of the male are more prominent. Eggs are laid in rotting logs or 10-15 cm below the soil surface near the taro plants. The

Fig. 7. Corm of *Colocasia esculenta* bored by *Papuana* beetle. (Dr. G.V.H. Jackson, South Pacific Commission, Nouméa)

Fig. 8. Damaged corms of *Colocasia esculenta* bored by *Papuana* beetles. (Dr. G.V.H. Jackson, South Pacific Commission, Nouméa)

larvae hatch in 11-16 days; they are white, curled and burrow through the soil probably feeding on rotting vegetation. The time from egg to adult is 120-210 days.

Alternative hosts

Angiopteris evecta, Canna indica, Cyrtosperma chamissonis, Ipomoea batatas, Musa sp., *Bandanas* spp., *Saccharum edule*, sugarcane, *Solanum tuberosum.*

Other beetles

Ligyrus spp. : the appearance and damage caused is similar to that of *Papuana* spp. The beetle occurs in Bolivia, Brazil, Colombia, French Guiana, Guyana, Mexico, Peru, Surinam, Trinidad and Venezuela.

Quarantine measures

- The unrestricted movement of taro between countries should not be permitted. If it is essential to import corms they should be washed free of soil, and fumigated or dipped in insecticide (carbaryl/malathion, white oil mixture) and treated with fungicide.
- Imports should preferably be in the form of sterile, pathogen-tested plantlets growing in tissue culture medium.

2. Taro planthopper, *Tarophagus proserpina*

Damage
The planthopper feeds on the sap of plants and heavy infestations can cause plants to wilt and become stunted. *Tarophagus* is the vector of the taro rhabdovirus (bobone virus).

Geographical distribution
Australia (Queensland), Hawaii, Indonesia, Japan (Ryukyu Islands), Malaysia, Philippines and almost all Pacific island countries (Anonymous, 1972; Anonymous, 1978).

Biology
The adult is 4 mm long, black with a broad white patch on the back of the thorax and abdomen (Fig. 9). There are short- and long-winged forms. Eggs are laid in the base of the petioles and in midribs of leaves. Young nymphs are creamy white; later predominantly black with white markings. From egg to adult takes about 18 days. Feeding and egg-laying punctures cause sap exudation which forms red encrustations on the plant (Anonymous, 1978; Jackson, 1980).

Fig.9. Taro Planthoppers, *Tarophagus proserpina.* (Dr. G.V.H. Jackson, South) Pacific Commission, Nouméa)

Alternative hosts
None

Quarantine measures
- Remove sheathing petiole bases until they are free of signs of feeding or egg-laying punctures, fumigate or dip in insecticide (carbaryl/malathion, white oil mixture) and treat with fungicide.
- Imports should preferably be in the form of sterile, pathogen-tested plantlets growing in tissue culture medium.

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Nematodes

Taro nematode, *Hirschmanniella miticausa*

Damage
Symptoms are usually only apparent when corms are harvested. Internally, a dry brown rot extends in narrow irregular bands upwards from the base of the corm, at first confined to vascular tissue (Fig. 10). The overall effect is that of uncooked meat, hence the pidgin name in Solomon Islands of 'miti-miti'. Often the basal parts of the corms are completely decayed by secondary soft rots.

Geographical distribution
Papua New Guinea and Solomon Islands (Jackson, 1980; Mortimer, Bridge and Jackson, 1981).

Biology
It is most likely that the nematode is spread on setts used for propagation. It is a pest of dryland taro but is especially serious in wetland cultivation. The epidemiology of the disease has not been studied, but Bridge, Mortimer and Jackson (1983) give a description of the nematode.

Fig.10. Symptoms of nematode attack by *Hirschmanniela miticausa* in corms of *Colocasia esculenta.* (Dr. G.V.H. Jackson, South Pacific Commission) Nouméa)

Alternative hosts
None known.

Other nematodes
In parts of Federated States of Micronesia and Palau the burrowing nematode, *Radopholus similis,* attacks the corms of the giant swamp taro and causes severe rot.

Quarantine measures
Strict quarantine measures should be observed to prevent further spread of this disease and movement of taro between countries should be limited to sterile, pathogen-tested plantlets growing in tissue culture medium.

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