

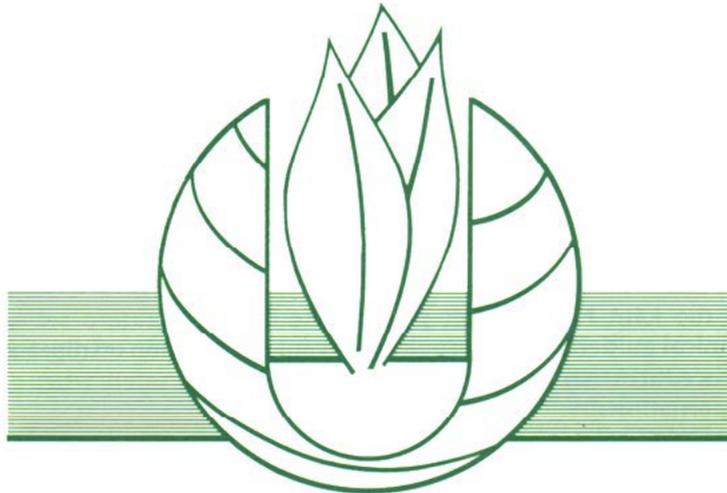


FOOD AND AGRICULTURE ORGANIZATION
OF THE UNITED NATIONS



INTERNATIONAL BOARD FOR
PLANT GENETIC RESOURCES

**FAO/IBPGR TECHNICAL GUIDELINES
FOR THE
SAFE MOVEMENT OF
SWEET POTATO GERMPLASM**



**Edited by
J.W. Moyer, G.V.H. Jackson and E.A. Frison**

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 intermediate 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 sweet potato germplasm:

Seed

- If it is not essential to move particular genotypes, and if they are available, then seeds should be preferred for the movement of sweet potato germplasm.
- Seeds should be selected from plants that appear healthy. They should be fumigated and treated with fungicide.
- Seeds should be germinated and seedlings grown under isolation or glass/screenhouse conditions in the recipient institution and seedlings should be observed periodically for virus symptoms. It is also advisable to perform serological tests with available antisera on random samples.

Vegetative propagating material

- Sweet potato germplasm, in vegetative form, should be transferred internationally as *in vitro* plantlets. A review of tissue culture techniques is given by Love, Rhodes and Moyer (1989).
- For the movement of *in vitro* cultures, neither antibiotics nor charcoal should be added to the medium.
- Therapy by meristem-tip culture, alone or in combination with thermotherapy, should be applied to all tissue culture material, preferably in the country of origin or in a third country (Frison and Ng, 1981). It is recommended to test the cultures for the presence of bacteria (see **Therapy** below).
- One to four nodes from the plantlets regenerated from meristem tips should be subcultured and maintained *in vitro* to prevent recontamination. The remainder (basal part of the plantlet) should be grown in an insect-free greenhouse for virus testing.
- Cultures should be checked for systemic bacterial and fungal contamination and for other pests before despatching and after receiving.

- All germplasm should be tested for the absence of viruses in the country of origin, in an intermediate quarantine centre, or in post-entry quarantine (see **Indexing** below).

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- Frison, E.A. and Ng, S.Y. 1981. Elimination of sweet potato virus disease agents by meristem tip culture. *Trop. Pest Manag.* **27**(4):452-454.
- Love, S.L., Rhodes, B.B. and Moyer, J.W. 1989 (second edition). *Meristem-tip culture and virus indexing of sweet potatoes*. International Board for Plant Genetic Resources, Rome.

Intermediate quarantine stations available for sweet potato*

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*This list is not exclusive but was developed by the meeting based on information given by the participants.

PESTS OF QUARANTINE IMPORTANCE

Viral diseases

Because of the great variability in symptoms due to plant genotype, plant age, environmental condition and the presence of virus complexes, symptoms on sweet potato, including the absence of symptoms, have little value for virus diagnosis. Visual inspection is therefore unreliable for virus identification and detection. Most viruses in sweet potato can only be reliably detected by grafting on to the indicator species *Ipomoea setosa*.

Several of the viruses which infect sweet potatoes are incompletely characterized. For convenience they have been divided into three categories according to the availability of detection techniques. Their principal characteristics are given in tables 1-3.

- Viruses that can be detected in *I. setosa* and for which antisera are available (Table 1). Four viruses from this category are described individually below.
- Viruses that can be detected in *I. setosa* but for which antisera are not yet available (Table 2).
- Virus and virus-like diseases that can not be detected in *I. setosa* (Table 3).

Therapy

Meristem-tip culture has been demonstrated to be effective for eliminating viruses in sweet potato and is recommended whenever germplasm is moved. Additional therapy measures, such as heat-therapy, may be necessary. This technique is fully described by Love, Rhodes and Moyer (1989). It is recommended, when clones are put in tissue culture, to routinely place the initial explants for 2 or 3 weeks on a medium that promotes the growth of bacteria (e.g. 40 g Trypticase Soy Agar in 1000 ml of water). Those meristems that remain free of bacterial growth are transferred to a medium allowing meristem growth (F. Quak, personal communication).

One to four nodes from the regenerated plant should be subcultured and maintained *in vitro* to prevent recontamination. The remainder (basal part) of the plant should be transplanted and grown in an insect-free greenhouse for virus testing.

Indexing

Leaves from plantlets regenerated from meristem-tips can be serologically assayed, as a preliminary screening, when the plantlets are subcultured. At this stage, negative results, using assays available, should not be interpreted as freedom from virus.

The basal part of the plantlet should be grown in an insect-free greenhouse until it has at least 10 nodes on the main stem. Two nodes from each sweet potato plant should

then be grafted on to *I. setosa*. The sweet potato plant should then be trimmed and allowed to regrow to a similar size and retested in the same manner. One of the nodes should be taken from the older part of the plant, below the regrowth. The method of grafting may be by either 'approach' or 'cleft' graft. However, a positive control such as sweet potato feathery mottle virus (SPFMV)-infected sweet potato should be included in each group to be tested to ensure that the method being used is successful. Both source and indicator plants should be grown in as near optimal conditions as possible to stimulate rapid and luxuriant growth. *I. setosa* should be held for a minimum of 4 weeks for observation of symptom expression. Symptomless *I. setosa* plants should be serologically assayed for SPFMV and sweet potato latent virus (SPLV). It is suggested that other available antisera also be used, and that the viruses tested for be specified. All plants (source and indicator) should be destroyed in accordance with local phytosanitary procedures. If all tests are negative, plantlets originated from the same meristem can be distributed.

Detection of the whitefly transmitted component of the sweet potato virus disease (SPVD) can not be done reliably in *I. setosa* at the present time. Plants should be sampled in a manner similar to that described above and then grafted to the TIB 8 tester clone of sweet potato developed by IITA. The tester clone is infected with a strain of SPFMV prior to grafting. The strain of SPFMV should be known to react synergistically with the whitefly component to cause the symptom of SPVD. Because of the occurrence of symptomless infection of SPFMV and SPLV, this tester clone should be indexed and a positive control included in each group of plants to be grafted.

1. Cucumber mosaic virus (CMV)

(cucumo virus group)

Symptoms

Stunting, chlorosis and yellowing of plants. CMV apparently only infects sweet potato plants previously infected with SPFMV.

Geographical distribution

CMV occurs worldwide, but is only reported in sweet potato from Israel and West Africa (Cohen, Loebenstein and Spiegel, 1988; Clark and Moyer, 1988).

Transmission

The virus is transmitted by vegetative propagation. It is also non-persistently transmitted by a large number of aphid species. Transmission to sweet potato requires the presence of SPFMV in the acceptor plant (Cohen, Loebenstein and Spiegel, 1988).

Particle morphology

Isometric, about 30 nm in diameter.

Indexing

The virus is mechanically transmissible to several *Ipomoea* species including *I. nil*, *I. purpurea*, *I. lacunosa* and *I. trichocarpa* as well as *Cucumis sativus* and *Nicotiana glutinosa*. *I. setosa* has not been successfully infected.

2. Sweet potato feathery mottle virus (SPFMV)

(potyvirus group)

Symptoms

Faint to distinct chlorotic patterns on the leaves, sometimes with pigmented borders. Some infected cultivars express internal (internal cork) or external (russet crack) necrosis on the roots. Infected plants may also be symptomless. Symptoms on *I. setosa* are shown in Fig. 1.

Geographical distribution

Worldwide (Clark and Moyer, 1988).

Transmission

Non-persistently transmitted by a large number of aphid species and also by vegetative propagation.

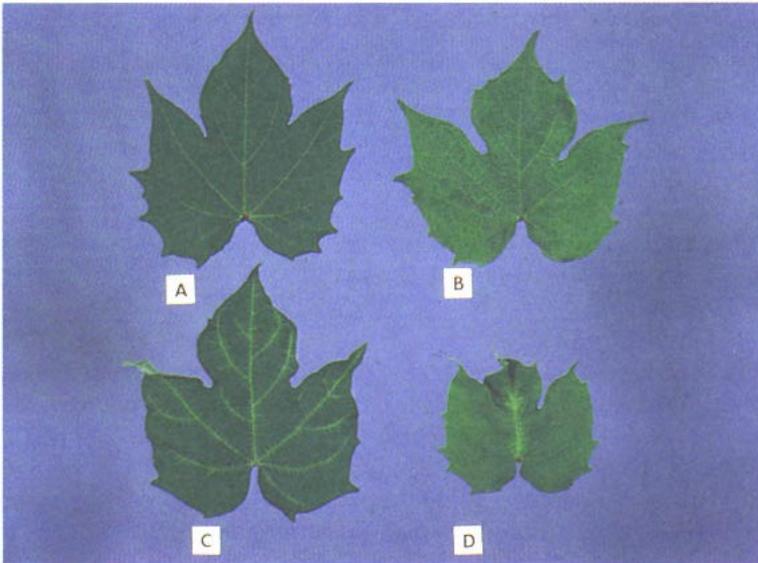


Fig. 1. Symptoms of SPFMV on infected (B-D) *I. setosa* leaves (A=healthy).
(Dr. J. Moyer, North Carolina State University, Raleigh)

Particle morphology

Flexuous rod with helical morphology, about 13 x 850 nm.

Indexing

Graft transmission to *I. setosa*. Diagnosis can be confirmed by serological tests of *I. setosa*.

3. Sweet potato latent virus (SPLV)

(potyvirus group)

Symptoms

Latent or mild chlorosis in sweet potato.. Symptoms on *I. setosa* are shown in Fig. 2.

Geographical distribution

Asia (Chung *et al.*, 1986; Liao *et al.*, 1979; Clark and Moyer, 1988).

Transmission

No known vector.

Particle morphology

Flexuous rod with helical morphology, about 13 x 750 nm.

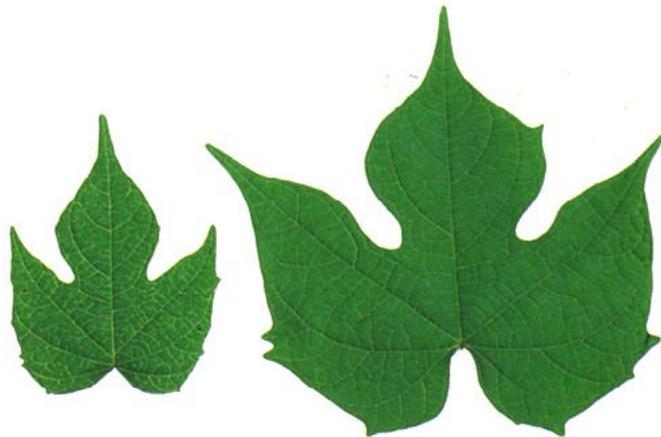


Fig. 2. Symptoms of SPLV on *I. setosa* (the leaf on the right is healthy). (Dr. S.K. Green, AVRDC, Shanhua)

Indexing

Graft transmission to *I. setosa*. Diagnosis can be confirmed by serological tests of *I. setosa*.

4. Sweet potato mild mottle virus (SPMMV)

(potyvirus group)

Symptoms

Infected sweet potatoes exhibit stunting and leaf mottling. Symptoms on *I. setosa* are shown in Fig. 3.

Geographical distribution

So far only reported from East Africa (Hollings, Stone and Bock, 1976).

Transmission

Efficiently transmitted by the whitefly *Bemisia tabaci* and by vegetative propagation material.

Particle morphology

Flexuous rod shaped particles, 800-900 nm in length.

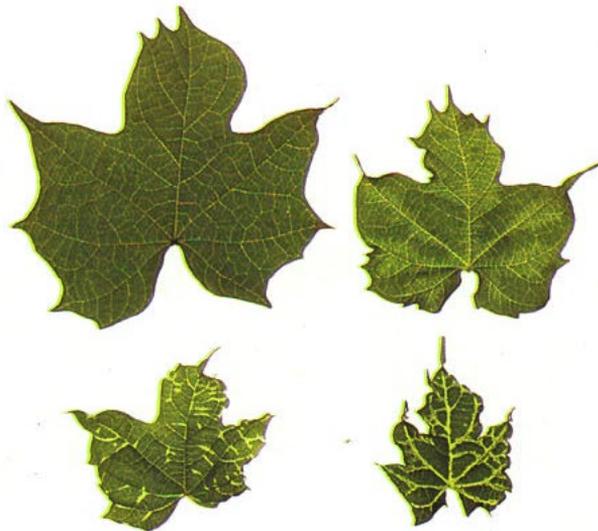


Fig. 3. Symptoms of SPMMV on infected *I. setosa* leaves (healthy leaf is at top left). (Dr. J. Moyer, North Carolina State University, Raleigh)

Indexing

Graft transmission to *I. setosa*. Diagnosis can be confirmed by serological tests of *I. setosa*.

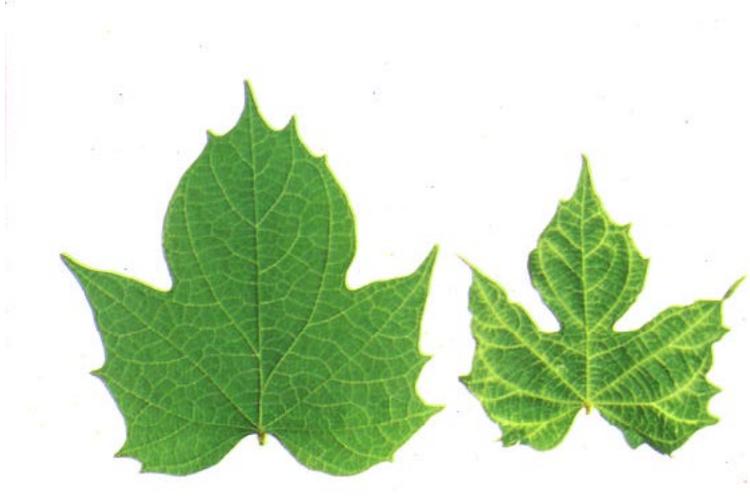


Fig. 4. Symptoms of SPV-II (see table 1) in *I. setosa* (the leaf on the left is healthy). (Dr. S.K. Green, AVRDC, Shanhua)

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Fig. 5. Symptoms of SPYDV (see table 1) on sweet potato (unknown variety).
(Dr. S.K. Green, AVRDC, Shanhua)

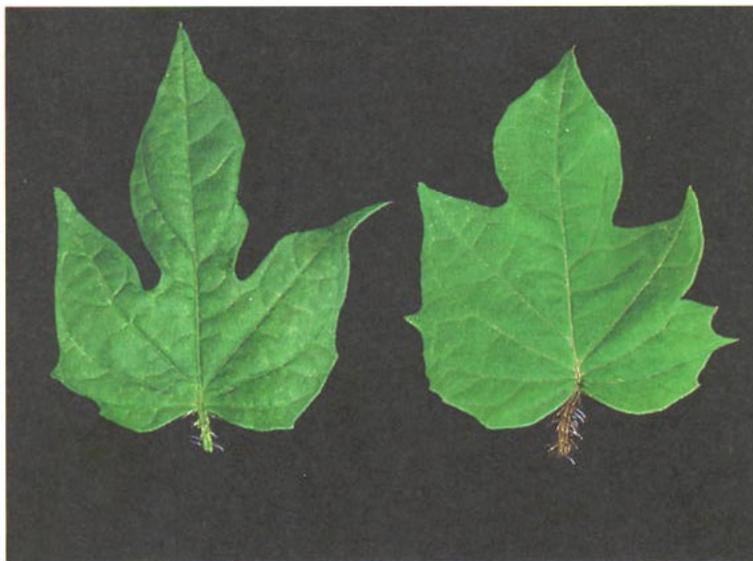


Fig. 6. Symptoms of SPYDV (see table 1) on *I. setosa* (the leaf on the right is healthy). (Dr. SK. Green, AVRDC, Shanhua)

Table 1. Viruses of sweet potato that can be detected in *Ipomoea setosa* and for which antisera are available

Designation	Distribution	Classification	Vector	Additional indicator plants	Reference/ contact person
Sweet potato feathery mottle virus (SPFMV)	Worldwide	potyvirus	aphid	—	1,2,9,10
Sweet potato virus II (SPV-II)	Taiwan	potyvirus	aphid	<i>N. benthamiana</i>	H. Rossel*, S. Green
Sweet potato latent virus (SPLV)	Asia	potyvirus?	unknown	<i>N. benthamiana</i>	7
Sweet potato mild mottle virus (SPMMV)	East Africa	potyvirus?	<i>Bemisia tabaci</i>	<i>N. tabacum</i> <i>N. glutinosa</i> <i>N. benthamiana</i>	1
Sweet potato ring-spot virus (SPRSV)	Papua New Guinea	nepovirus?	unknown	—	A. Brunt*
Sweet potato caulimo-like virus (SPCLV)	widespread	?	?	<i>N. megalosiphon</i>	4, A. Brunt*
Sweet potato yellow dwarf virus (SPYDV)	Taiwan	potyvirus?	<i>Bemisia tabaci</i>	<i>G. globosa</i> <i>D. stramonium</i> <i>Cassia occidentalis</i>	3, S. Green*

* information generously provided for this report prior to publication



Fig. 7. Symptoms of sweet potato leafcurl virus (see table 2) on *I. setosa*.
(Dr. H.W. Rossel, IITA, Ibadan)



Fig. 8. Symptoms of 'unknown sweet potato virus' (see table 2) in *I. setosa*.
(Dr. J. Moyer, North Carolina State University, Raleigh)

Table 2. Viruses of sweet potato that can be detected in *Ipomoea setosa*, but for which no antisera are available

Designation	Distribution	Classification	Vector	Reference/ contact person *
Reo-like virus	Asia		?	A. Brunt
CIP-Isolates (C2-C6)	Unknown		?	L. Salazar
Sweet potato leafcurl virus (SPLCV)	Japan, Nigeria, Taiwan		<i>Bemisia tabaci</i>	3,13, S. Green, H. Rossel
Sweet potato mosaic virus (SPMV)	Taiwan			S. Green
Sweet potato vein mosaic virus (SWMV)	Argentina	potyvirus	aphid	11 (culture lost)
Unknown virus (not mechanically transmitted)	Puerto Rico		?	J. Moyer
Iilar-like virus	Guatemala		?	J. Moyer

* information generously provided for this report prior to publication



Fig. 9. Three types of symptoms of SPVD, a complex of SPFMV and a whitefly-transmitted component (see table 3), on sweet potato. (Dr. H.W. Rossel, IITA, Ibadan)

7. Liao, C.H., Chien, P.C., Chung, M.L., Chiu, R.J. and Han, Y .H. 1979. A study of a sweet potato virus disease in Taiwan. 1. Yellow spot virus. *J. Agric. Res. China* **28**:127-138.
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Table 3. Virus and virus-like diseases/agents of sweet potato that can not be detected in *Ipomoea setosa*

Designation	Distribution	Vector	Detection	Reference/ contact person *
Whitefly-transmitted component of sweet potato virus disease (SPVD)	Africa, Taiwan?	<i>Bemisia tabaci</i>	TIB 8 sweet potato infected with SPFMV	H. Rossel, 12
Chlorotic leaf distortion agent	United States		none available	C. Clark
Cucumber mosaic virus (CMV)	widespread	aphid	<i>N. glutinosa</i> <i>Cucumis sativus</i>	5

* information generously provided for this report prior to publication

Mycoplasmal disease

Sweet potato little leaf, or witches' broom disease

Cause

Mycoplasma-like organism (MLO).

Symptoms

Vein-clearing of otherwise healthy leaves is the first sign of infection, followed by the development of progressively smaller, chlorotic leaves sometimes rolled upwards at the margins, puckered and in some cultivars more rounded than normal. Diseased stems are short, erect, and multibranched due to growth of normally dormant axillary buds, giving a bushy appearance, further increased by gradual shortening of internodes to 1 cm or less (Figs. 10-12). Storage roots are either absent or pencil-thin, amid a mass of short, thin, extensively branched roots.

Geographical distribution

Federated States of Micronesia (Yap state), Japan (Ryukyu Islands), Korea, New Caledonia, Niue, Palau, Papua New Guinea, Solomon Islands, Taiwan, Tonga and Vanuatu (Jackson and Zettler, 1983; Jackson, Pearson and Zettler, 1984; Yang, 1969).



Fig. 10. Symptoms caused by MLO infection on cv. Tainung 66 (the leaves on the right are healthy). (Dr. S.K. Green, AVRDC, Shanhua)



Fig. 11. Proliferation of sweet potato shoots caused by MLO infection (plants at the top are healthy). (Dr. G.V.H. Jackson, South Pacific Commission, Nouméa)



Fig. 12. Symptoms caused by MLO infection on unknown sweet potato cultivar. (Dr. S.K. Green, AVRDC, Shanhua)

Transmission

Orosius lotophagorum ryukyuensis (in Pacific countries) and *Nesophrosyne (Orosius) ryukyuensis* (in Japan) are vectors. The MLO is not seedborne.

Alternative hosts

Vinca rosea and wild *Ipomoea* spp.

Therapy

Only meristem-tip culture is efficient (Green, Luo and Lee, 1989). Tetracycline will give remission of symptoms but will not eliminate the MLO from the plants.

Indexing

Vines should be tested by graft-inoculation to *Ipomoea setosa*; symptoms may take 6 months or more to develop. Plants that index negatively should be retested, and further checked by fluorescent antibody (Dale, 1988) or DNA-specific fluorescent dye (DAPI) (Pearson, Keane and Thiagalingham, 1984).

References

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Bacterial diseases

Unnamed disease

Cause

Erwinia chrysanthemi, *Streptomyces ipomoea* and *Pseudomonas batatas*. Although these pathogens occur primarily on the roots, *E. chrysanthemi* and *P. batatas* may also move into the vascular tissues of above-ground parts of the plant. For details on biology and symptoms refer to the APS compendium on sweet potato diseases (Clark and Moyer, 1988).

Geographical distribution

E. chrysanthemi and *S. ipomoea* pathogens are probably universal, whereas *P. batatas* has so far only been reported from China (Clark and Moyer, 1988).

Therapy

None is recommended. The use of antibiotics in the tissue-culture medium is not advised because of their inhibitory effect on the plant tissue. However, non-inhibitory antibiotics could be used prior to meristem excision. Rifamycin seems not so harmful when stem cuttings are put in a solution containing such antibiotics (Dr. S.K. Green, personal communication).

Indexing

It is recommended, when clones are put in tissue culture, to routinely place the initial explants for 2 or 3 weeks on a medium that promotes the growth of bacteria (e.g. 40 g Trypticase Soy Agar in 1000 ml of water). Those meristems that remain free of bacterial growth are transferred to a medium allowing meristem growth (F. Quak, personal communication).

Quarantine measures

Material for tissue culture should be taken from apparently disease-free stem cuttings. Only tissue-cultured and bacteria-tested material should be distributed.

Reference

Clark, C.A. and Moyer, J.W. 1988. *Compendium of Sweet Potato Diseases*. APS Press, St. Paul.

Fungal diseases

Sweet potato scab

Cause

Elsinoe batatas

Symptoms

Small, brown, scabby areas mostly among the leaf midrib and veins and on the petioles, 1-5 mm at first, later joining together for several centimetres (Figs. 13 and 14). Numerous pinpoint size spots occur in patches between the veins. Severe distortion of leaves occurs as early infection of veins prevents normal leaf expansion. Leaves are small, curled, with deeply torn edges and petioles are short and twisted. Tubers are not infected (Clark and Moyer, 1988; Jackson and McKenzie, 1989).

Geographical distribution

Brazil, Brunei, China, Indonesia (Irian Jaya), Japan, Malaysia, Philippines, Taiwan and the following Pacific island countries: Cook Islands, Federated States of Micronesia (excluding Kosrae), Fiji, French Polynesia, Guam, New Caledonia, Niue, Palau, Papua New Guinea, Solomon Islands, Tonga and Vanuatu (Anonymous, 1982).



Fig. 13. Scab lesions of *Elsinoe batatas* along midrib and veins on the under surface of a sweet potato leaf. (Dr. G.V.H. Jackson, South Pacific Commission, Nouméa)



Fig. 14. Sweet potato variety susceptible (right) to *Elsinoe batatas* (resistant variety on the left). (Dr. G.V.H. Jackson, South Pacific Commission, Nouméa)

Biology

Minute spores are produced in the scabby areas and spread in rain-splash to healthy shoots. Temperatures between 13°C and 26°C and high rainfall favour the disease. The fungus is assumed to survive in debris between crops and is spread to new areas in infected vines.

Alternative hosts

None known.

Quarantine measures

- The movement of vines and tubers between countries should be avoided.
- Preference should be given to importing seeds or sterile, pathogen-tested, tissue cultures growing in a tissue-culture medium.

Reference

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Insect pests

1. Sweet potato weevils, *Cylas formicarius* and *C. puncticollis*

Damage

Adults (Fig. 15) cause minor damage by scraping off surface layers of roots, leaves, petioles and stems as they feed. Larvae make long, twisting, frass-filled tunnels, and even undamaged parts are spoilt by the presence of offensive terpene odours produced in response to attack. Vines are bored; they may be killed by *C. puncticollis* attack. Damage to roots (Fig. 16) continues in storage (Schalk and Jones, 1985).

Geographical distribution

C. formicarius is widely distributed; it occurs in Africa, Asia, the Caribbean, in parts of North and South America and the Pacific (Anonymous, 1970a). *C. puncticollis* is confined to Africa (Anonymous, 1970b).

Biology

Adults, 6-7 mm long, have blue-black wing covers. Legs, thorax and antennae are reddish-brown (*C. formicarius*) or black (*C. puncticollis*). Eggs are laid singly, at the base of vines, or in roots. After 5-8 days, white, legless larvae hatch, feed inside the roots or vines for 15-20 days and then pupate. Adults emerge from the pupal cases about 7



Fig. 15. Adult potato weevil, *Cylas formicarius*. (Dr. G.V.H. Jackson, South Pacific Commission, Nouméa)



Fig. 16. Feeding damage of sweet potato weevil on storage roots. (Dr. N.S. Talekar, AVRDC, Shanhua)

days later but remain inside the plant for a further 6-9 days. Egg-laying begins 2-3 days after emergence and lasts throughout the 70-90 days of adult life.

Alternative hosts

Wild *Ipomoea* spp.

Quarantine measures

- The movement of vines and roots between countries should be avoided
- Preference should be given to importing seeds or sterile, pathogen-tested, tissue cultures growing in a tissue-culture medium.
- If it is essential to move vegetative propagating material between countries, young vines should be fumigated or dipped in insecticide (carbaryl/malathion, white oil mixture), and treated with a fungicide.

2. West Indian sweet potato weevil, *Euscepes postfasciatus*

Damage

Larvae tunnel into roots and vines.

Geographical distribution

Australia, Central and South America, Hawaii, the West Indies, Japan (Ryukyu Islands) and the following Pacific island countries: Cook Islands, Fiji, French Polynesia, Guam, New Caledonia, Tonga, Vanuatu, Wallis and Futuna (Anonymous, 1973; Macfarlane and Jackson, 1989).

Biology

Adults are 4-5 mm long, greyish-brown with a white mark near the rear of the body. Eggs laid in the roots hatch within 7 days. Larvae are 5 mm long when full grown at 25-30 days. They are legless, white with a yellowish head and reddish-brown mandibles. Pupation lasts about 7 days, and after emergence, adults remain inside the pupal case until integuments harden (Anonymous, 1978).

Alternative hosts

Wild *Ipomoea* spp.

Quarantine measures

Same as for *Cylas formicarius*.

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