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Seed Health Testing and Phytosanitary Procedures for Tropical Forages

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I. INTRODUCTION

According to Kahn (1989), the word "quarantine" originated from the Latin "quadraginata" and the Italian "quarantina", applied to the 40-day period of isolation required for a ship (passengers and cargo) to remain anchored in port of arrival if the ship arrived from a country where certain epidemic diseases were known to occur. This practice was started to allow the development of symptoms before any passengers debarked.

Plant quarantines regulate the entry of plants and their products, soil, microbial cultures, and commodities in order to protect agriculture and the environment from avoidable pests and pathogens. Organisms of quarantine importance may include pests or pathogens that governments consider threats to the agriculture and environment of countries. These organisms are either foreign to a country or include foreign strains and races of already existing organisms.

In the case of forage legumes and grasses this becomes very complicated by the following factors: **1.** there are numerous plant genera and species, **2.** there are a number of pathogens which are capable of infecting these wild plants, **3.** the lack of appropriate and complete documentation of these pathogens, **4.** lack of information and scientific data on the biology, epidemiology, economic importance, complete geographic distribution, etc. of the documented pathogens.

Despite these complications and difficulties, careful tests and treatments should enable us to ensure safe movement of forage germplasm.

Testing, screening, disinfection and seed decontamination are procedures to be considered in phytosanitary programs.

II. REVIEW OF LITERATURE ON SEED-BORNE PATHOGENS OF TROPICAL FORAGE PLANTS

There are many seed-borne pathogens in Gramineae and Leguminosae which are frequently transmitted. Seed transmission of pathogens depends basically on the inherent features of both the host and the pathogen. Members of a plant family transmit groups of related pathogens through seeds; e.g., smut fungi in Graminae, viruses or *Colletotrichum* spp. in Leguminosae.

In the family Gramineae, there is a wide range of seed-borne pathogens such as the smuts, ergots, and seed gall nematodes.

Seed-borne diseases are common in Leguminosae and probably far more severe than in other plant families.

There is very little published information on seed-borne pathogens of tropical forage plants. *Colletotrichum gloeosporioides* and *C. truncatum* were reported to significantly reduce emergence, survival, and root and shoot dry weight of *Stylosanthes hamata* as seed-borne pathogens (Lenné and Sonoda, 1979). *C. gleosporioides* can also be a seed-borne pathogen of *Centrosema pubescens* and *Pueraria phaseoloides* (unpublished documents of Dr. J. Lenné).

Rhizopus stolonifer, the causal agent of seedling blight and inflorescence diseases of *Stylosanthes* spp., was reported to be the most common fungus associated with seeds of *S. hamata* in Florida (Lenné and Sonoda, 1978). In germination tests, this organism affected seedling emergence and survival.

Seed-borne pathogens of other tropical forage legumes have been reported. *Pseudomonas florescens*, the cause of severe pod rot of *Leucaena leucocephala*, and bacterial leaf spot and dieback of *Centrosema* spp., has been reported to be seed-borne (Lenné *et al.*, 1981a, 1981b). *Pseudomonas syringae* which causes leaf spot disease of *Centrosema pubescens* could be seed-borne (Duveiller, 1987).

Seed-borne or seed associated pathogens of tropical forage legumes.

Host	Pathogen	Country
*Arachis***	*Cercospora arachidicola*	Brazil, Colombia
	Cercospora canescens	Malawi
	Cercospora personata	Brazil, Colombia
	Cochliobolus lunatus	Nigeria
	Colletotrichum capsici	Nigeria
	Colletotrichum gloeosporioides	Brazil, Colombia, Australia
	Colletotrichum truncatum	Colombia
	Fusarium spp.	Colombia
	Leptosphaerulina arachidicola	Brazil, Colombia, Costa Rica, Ecuador, Peru, USA
	Mycosphaerella arachidis	Nigeria
	Myrothecium roridum	India
	Peanut mottle virus	Brazil, Colombia
	Puccinia arachidis	Brazil
	Periconia spp.	Colombia
	Rhizoctonia solani	Brazil, Colombia, Costa Rica, Peru
	Sphaceloma arachidis	Brazil, Colombia
Centrosema	*Cercospora canescens*	Australia, Barbados, Bolivia, Brazil, Colombia, Costa Rica, Cuba, Ecuador, French Guyana, Malaysia, Panama, Peru, Sudan, USA, Venezuela
	Colletotrichum gloeosporioides	Australia, Barbados, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, Peru, USA, Venezuela
	Colletotrichum truncatum	Colombia, USA
	Mosaic viruses	Australia, Brazil, Colombia, Cuba
	Pseudomonas flourescens	Colombia
	Pseudomonas syringae	Burundi
	Rhizoctonia sp.	Australia, Brazil, Colombia, Costa Rica, Ecuador, French Guyana, Ghana, Indonesia, Malaysia, Papua New Guinea, Peru, Sierra Leone, Seychelles, Solomon Islands, USA, Vanuatu

Host	Pathogen	Country
Desmodium sp.	*Cercospora* sp.	Australia, Barbados, Brazil, Canada, Colombia, Dominican Republic, El Salvador, Ghana, India, Jamaica, Kenya, Malaysia, Pakistan, Papua New Guinea, Tanzania, USA, Venezuela, Zimbabwe
	Cladosporium cladosporioides	Australia
	Cladosporium sp.	India, Cuba
	Colletotrichum gloeosporioides	Australia, Bolivia, Brazil, Colombia, Ecuador, Peru, USA, Venezuela
	Colletotrichum truncatum	Colombia, USA
	Desmodium mosaic virus	USA
	Rhizoctonia sp.	Brazil, Colombia, Costa Rica, El Salvador, Malaysia, Peru
	Synchytrium desmodii	Colombia, Ecuador, Tanzania
Pueraria sp.	*Cercospora* sp.	Cambodia, Cuba, Malaysia, Philippines, Puerto Rico, Sierra Leone, Taiwan, Tanzania
	Cladosporium sp.	Sierra Leone
	Colletotrichum gloeosporoides	Brazil, Colombia, Ecuador, Peru, Puerto Rico, Venezuela, Virgin Islands
	Drechslera sp.	Malaysia
	Fusarium sp.	Papua New Guinea, Sierra Leone
	Mycosphaerella puerariicola	Cambodia, Hong Kong, Japan, Malaysia, Philippines, Singapore, Solomon Islands, Taiwan, USA
	Pseudomonas phaseolicola	USA
	Rhizoctonia sp.	Brazil, Colombia, Costa Rica, Ecuador, Malaysia, Peru, Sierra Leone, Solomon Islands
Stylosanthes	*Ascochyta* spp.	Colombia
	Bipolaris spp.	Colombia
	Botrytis cinerea	Australia, Colombia, Zimbabwe
	Cladosporium spp.	Australia, Brazil

Host	Pathogen	Country
Stylosanthes	*Colletotrichum gloeosporioides*	Angola, Australia, Belize, Bolivia, Botswana, Brazil, Colombia, Costa Rica, Cuba, Ecuador, Ethiopia, French Guyana, India, Ivory Coast, Kenya, Malaysia, Malawi, Mexico, Mozambique, Nigeria, Panama, Papua New Guinea, Peru, Solomon Islands, Tanzania, Thailand, Venezuela, Zaire, Zambia, Zimbabwe, USA
	Curvularia spp.	Australia, Colombia, USA
	C. truncatum	Australia, Bolivia, Brazil, Colombia, Ecuador, Ethiopia, Nigeria, Peru, Thailand, USA, Venezuela
	Eurotium spp.	Colombia
	Fusarium spp.	Australia*, Brazil, Colombia, Malaysia, Nigeria
	Gloeocercospora spp.	Colombia
	Leptosphaerulina spp.	Colombia, Australia
	Phoma sorghina	Australia, Colombia, Ivory Coast, Nigeria
	Phomopsis spp.	Colombia, Bolivia, Nigeria, Malaysia, Trinidad,
	Potyvirus	Colombia
	Rhizopus stolonifer	Colombia, India, USA
	Rhizoctonia spp.	Australia, Colombia, Malaysia, Papua New Guinea
	Sclerotinia sclerotiorum	India, Zimbabwe
Zornia sp.	*Colletotrichum gloeosporioides*	Brazil, Colombia, Costa Rica, Ecuador, Panama, Peru, USA
	Colletotrichum truncatum	Bolivia, Brazil, Colombia, Ecuador, Peru, USA, Venezuela,
	Corynebacterium flaccumfaciens	Colombia
	Fusarium sp.	Colombia
	Phoma sorghina	Colombia
	Rhizoctonia solani	Brazil, Colombia, Peru
	Sphaceloma zomiae	Brazil, Colombia, Peru, Venezuela,

* Several species of *Fusarium* reported.

** The pathogens are not necessarily seed-borne or seed associated. These can be transported through planting materials such as cuttings.

Source (fungal pathogens): Lenné, J.M. 1990. A world list of fungal diseases of tropical pasture species. CAB International, Wallingford, U.K.

Seed-borne or seed-associated pathogens of tropical forage grasses.

Host	Pathogen	Country
Andropogon	*Balansia*	Bengal, China, India, Mexico, Nigeria, Philippines, Puerto Rico, Sierra Leone, West Indies, Zambia,
	Cerebella andropogonis	Burma, Cuba, Ghana, Jamaica, Romania, Sierra Leone, Sri Lanka, Uganda, Zambia, Zimbabwe
	Claviceps, ergot	Bengal, Colombia Kenya, USA,
	Curvularia sp.	Colombia, Nigeria, Sudan
	Phoma sorghina	Colombia, Sri Lanka
	Sorosporium	Argentina, Cambodia, Colombia, Congo, India, Israel, Morocco, Sierra Leone, South Africa, Sri Lanka, USA
	Sphacelia sorghi, ergot	Ethiopia, Ghana
	Sphacelotheca spp.	Bolivia, Brazil, China, Gambia, India, Malawi, Mexico, Pakistan, Sierra Leone, Sudan, Tanzania, Uganda, USA, Venezuela, Zambia, Zimbabwe
	Tolyposporella, smut	Brazil, Dominican Republic, Mexico, Puerto Rico
	Tolyposporium, smut	Nigeria, India
	Tolysporum, smut	India
	Ustilago, smut	Brazil, Congo, Ghana, India, Malawi, Mexico, Nigeria, Sierra Leone, Venezuela, West Africa
Brachiaria sp.	*Alternaria alternata*	Kenya, Pakistan
	Balansia sp., false smut	Brazil, Fiji, India, Zambia
	Botryodiplodia	Colombia
	Ceratocystis paradoxa	Colombia
	Cercospora sp.	Australia, Bengal, Botswana, Ethiopia, French Guyana, Kenya, Mali, Tanzania, Uganda, Zambia, Zimbabwe
	Cerebella andropogonis, false smut	Bengal, Burma, Kenya, South Africa, Sudan, Tanzania, Uganda, USA, Zambia, Zimbabwe

Host	Pathogen	Country
Brachiaria sp.	*Cladosporium* sp.	Brazil, Colombia, Ethiopia, Pakistan, Uganda
	Claviceps sp., ergot	Australia, Bengal, Ethiopia, India, Kenya, Malawi, Tanzania, Zambia, Zimbabwe
	Curvularia	Colombia, Fiji
	Ephelis sp.	Australia
	Fusarium acuminatum	Colombia
	Fusarium heterosporum	Ethiopia, Kenya, Tanzania, Uganda, Zambia, Zimbabwe,
	Fusarium sp.*	Brazil, Colombia, Ecuador, French Guyana, Peru, Zambia
	Guineagrass mosaic virus***	Africa, Brazil, Colombia, Peru
	Melanotaenium brachiariae, smut	India
	Phoma sorghina	Colombia
	Pyricularia oryzae	Colombia, Fiji, India, Nepal, Malaysia, Philippines, Thailand
	Rhizoctonia solani	Brazil, Colombia, Costa Rica, Ecuador, Ethiopia, Guadalcanal, India, Malaysia, Peru, Solomon Islands
	Rhizopus stolonifera	Colombia
	Sorosporium brachiariae, smut	Zambia, Zimbabwe
	Sorosporium cryptum, smut	Australia
	Sorosporium sp., smut	Malawi, USA
	Sphacelia sp., ergot	Burma, Sudan, Uganda, Zambia
	Sphacelotheca serrata, smut	Malawi
	Ustilago operta, smut	India, Pakistan, Sudan
	Ustilaginoidea virens, false smut	Kenya, Malawi, Peru, Sudan, Tanzania, Zambia, Zimbabwe,
	Xanthomonas sp.**	Colombia, French Guyana
Panicum	*Balansia*	Brazil, Costa Rica, Guinea, India, Jamaica, Malaysia, Nicaragua, Panama, Sierra Leone, Trinidad, Venezuela
	Bipolaris	Australia, China, India, South Africa, USA, Venezuela

Host	Pathogen	Country
Panicum	*Cercospora*	Australia, Bolivia, Brazil, Colombia, Costa Rica, Cuba, Ecuador, El Salvador, Ethiopia, Fiji, French Guyana, Ghana, Guadalupe, Guatemala, India, Ivory Coast, Jamaica, Kenya, Malaysia, Mexico, Nicaragua, Nigeria, Panama, Papua New Guinea, Peru, Puerto Rico, Sierra Leone, Sudan, Tanzania, Thailand, Togo, Trinidad, Uganda, USA, Vanuatu, Venezuela, Virgin Islands, Zambia, Zimbabwe,
	Cerebella	Brazil, Dominican Republic, Ghana, India, Jamaica, Nigeria, Malawi, Puerto Rico, Sierra Leone, South Africa, Sudan, Tanzania, Venezuela, Virgin Islands, Zambia, Zimbabwe,
	Cochliobolus	Australia, Colombia, Cuba, Egypt, French Guyana, India, Japan, Jamaica, Nigeria, Papua New Guinea, Peru, Sierra Leone, Solomon Islands, USA, Zambia, Zimbabwe
	Cladosporium	Cyprus, Bolivia, Sierra Leone, Sudan, Venezuela, Zambia
	Claviceps	Australia, Bengal, Brazil, Colombia, Costa Rica, French Guyana, India, Kenya, Mauritius, Peru, Puerto Rico, Sierra Leone, Sudan, Tanzania, Venezuela, Virgin Islands, Zambia, Zimbabwe
	Fusarium	Bengal, Brazil, Cameroon, Colombia, Costa Rica, Ghana, Kenya, Malawi, Peru, Puerto Rico, Tanzania, Venezuela, Zambia, Zimbabwe
	Helminthosporium	Australia, Guadalupe, India, Malaysia
	Magnaporthe grisea	Australia, Dominican Republic, Egypt, India, Nigeria, Malaysia, Puerto Rico, Tanzania, USA, West Africa, Zambia, Zimbabwe,
	Phoma sorghina	Bolivia, Brazil, China, Ethiopia, India, Ivory Coast, Nigeria,

Host	Pathogen	Country
Panicum	*Sorosporium*	Angola, Australia, Bengal, Botswana, Congo, Cyprus, China, Ghana, India, Israel, Malawi, Northeast Africa, South Africa, Sri Lanka, Sudan, Tanzania, USA, Zimbabwe,
	Sphacelia sp.	Australia, Bengal, Brazil, Colombia, Costa Rica, Jamaica, Kenya, Malawi, Peru, Venezuela, Zambia
	Sphacelotheca	Australia, Bulgaria, Canada, Czechoslovakia, India, Poland, Yemen, Yugoslavia, Romania, USA, USSR
	Tilletia	Belize, Bolivia, Brazil, Canada, Cameroon, Colombia, Congo, Costa Rica, Cuba, Ecuador, Ghana, India, Ivory Coast, Kenya, Malawi, Mauritius, Mexico, Natal, Nigeria, Pakistan, Panama, Philippines, South Africa, Sri Lanka, Sudan, Tanzania, Uganda, Venezuela, Zambia, Zimbabwe
	Ustilaginoidea	Burma, Congo, Malaysia, Philippines, Puerto Rico, Tanzania, Trinidad & Tobago, Sierra Leone
	Ustilago	Argentina, Brazil, Bulgaria, Canada, Czechoslovakia, England, Guatemala, India, Italy, Kenya, Mauritius, Mozambique, Nigeria, Romania, Uganda, Yugoslavia, Sri Lanka, Sudan, Thailand, Trinidad, USA, Zimbabwe,

* Several species of *Fusarium* reported.
** Potential to be seed-borne.
*** Not seed-borne, but can be introduced through tissue culture or vegetative materials.

Source (fungal pathogens): Lenné, J.M. 1990. A world list of fungal diseases of tropical pasture species. CAB International, Wallingford, U.K.

III. SEED DISINFECTION OR DECONTAMINATION

Seed treatment for eradication of seed-borne pathogens can be used as a precaution in quarantine only if careful consideration is given to the limitations of such procedures. In quarantine, no residual inoculum must remain after treatment. The primary justification for seed treatment as a quarantine precaution is its use as an additional safeguard to kill undetected trace amounts of inoculum in apparently healthy seed lots. **Another acceptable possibility would be to treat seed which carry low amounts of inoculum, in order to save particularly valuable germplasm material.**

A number of countries require that certain seeds and / or propagative materials be treated with broad spectrum fungicides or specific fungicides as a condition of entry. Some of the fungicides most cited are thiram, benomyl, captan and vitavax.

IV. SEED HEALTH TESTING

Seed health testing is conducted to provide information for the following purposes:

1. Seed inspection for quarantine purposes
2. Seed quality evaluation for planting value
3. Seed certification purposes

i. Methods of detection of seed-borne fungi and bacteria

There are several methods of seed testing. Usually there are a number of microorganisms carried in a seed lot, and each of these may have requirements for its growth. The method to be employed will depend mostly on the purpose of the test, the particular pathogen, and the type of seed.

Seed health testing procedures (adopted from Neergaard, 1977)

METHOD	APPLICATION
Direct inspection Examination of the dry seed, with impurities, using a hand lens or, a stereo-microscope. Seeds may be submerged in water to release spores and facilitate detection.	Sclerotia of fungi, smut balls, nematode galls, infected plant debris; e.g., *Sclerotinia sclerotiorum, Botrytis cinerea, Claviceps purpurea.* Seeds discolored or with lesions produced by fungi, bacteria, or viruses, e.g., anthracnose fungi, some Xanthomonads, viruses such as soybean mosaic virus in leguminous seed.
Examination of suspension from washings of seed An electrical mechanical shaker can be used to obtain standardized washings. Samples of the suspension are examined under compound microscope.	Covered smuts, e.g., *Tilletia* spp. in monocots, oospores of certain downy mildews; quick orientation on the presence of other fungi that must be adequately detected by incubation procedures, e.g., *Drechslera* spp.
Whole-embryo count method Soaking grains overnight in 10% NaOH at 22 C, then washing with warm water through sieves of decreasing mesh size. Embryos finally cleared in lactophenol.	Loose smuts of monocots
Blotter method Seeds are incubated on water-moistened blotter, usually for 7 days at 20 C. Sporulation of fungi is stimulated by near-ultra-violet (NUV) irradiation, standard 12/12-hr light/dark cycle. Petri dishes are usually used as containers. To allow penetration of the NUV, plastic or pyrex glass containers should be used. Sometimes blotters are soaked in 0.1-0.2% 2,4-D solution to counteract seed germination, thus aiding recording.	The method is used most commonly for detecting a wide-range of Fungi Imperfecti, including different spp. of *Acremonium, Acroconidiella, Alternaria, Ascochyta, Botrytis, Cercospora, Colletotrichum, Diplodia, Drechslera, Fusarium, Macrophomina, Myrothecium, Phoma, Phomopsis, Septoria,* and others, and for practically all kinds of seed, including cereals, grasses, ornamentals, forest seeds, and vegetables.
Agar plate method The seeds are plated in Petri dishes on nutrient agar, in particular malt extract agar, potato-dextrose agar. Some selective media are available for specific tests. Light treatment as for the blotter test. Incubation for 5-7 days.	Although slowly growing fungi cannot be adequately detected, the procedure is relatively sensitive for revealing minor amounts of inoculum.

METHOD	APPLICATION
Freezing method A modified blotter method. After 1-2 days at 10-20 C, according to specifications, incubation for some hours or for 1 day at -20 C, then at 20 C in NUV light for 5-7 days.	Sometimes preferred for detecting certain fungi, e.g., *Phoma, Septoria, Alternaria.*
Ordinary seedling symptom test The seed is sown in autoclaved soil, sand, or similar material and placed under normal daylight conditions for observation of symptoms.	Often used for detecting seedling symptoms which reveal pathogens rather than fungi to be identified. Useful for detecting seedling pathogens.
Water-agar seedling symptom test The seeds are sown on water agar, in 16-mm test tubes, one seed per tube, or in microculture plastic plates or Petri dishes. They are placed under daylight conditions, e.g., 12/12-hr cycle of artificial daylight and darkness. Seedlings are inspected for symptoms, healthy seedlings may be transplanted for further post-entry quarantine cultivation.	Can be used for many kinds of seed as an economical procedure which,in test tubes, secures separation of healthy and infected seedlings. Has been used for detecting *Drechslera, Septoria, Macrophomina*; and other pathogens and hosts.
Indicator test, inoculation methods A standard technique for identification of viruses but also used for detection of trace amounts of pathogenic bacteria, e.g., by hypodermic injection of indicator plants with material from seeds slightly infected by the pathogen under test.	Used for detection of *Xanthomonas* in beans, crucifers, etc.
Phage-plaque method Maceration of the seed to be tested followed by incubation by for 24 hr to enable multiplication of bacteria. Samples of this material transferred to sterile flasks, and a standard suspension of phage particles is added. Samples of this mixture are plated immediately and after 6-12 hr on plates with the indicator bacterium. Presence of homologous bacteria is indicated by significant increase in number of phage particles in the second plating.	Used for *Pseudomonas phaseolicola* and *Xanthomonas phaseoli* in French bean, *Xanthomonas vesicatoria* in tomato.

METHOD	APPLICATION
Serological methods An antiserum must be provided and the tests may follow different procedures: the slide agglutination test, the tube precipitin test, the micro-precipitin test, the gel double- diffusion test, the latex flocculation test, the immunofluorescence test.	Used for different seed-borne viruses, and may be used for any pathogen for which an antiserum is provided.

ii. Selection of tests

The test to be used should be capable of revealing the widest possible range of pathogens. The procedures can be combined in different ways. For instance, healthy-looking seedlings can be selected from the blotter and/or agar plate tests and transplanted into soil, sand, or another suitable medium for further growth and observation. The combination of procedures saves time and seed.

In addition to generalized procedures, it may be necessary to include specialized tests to detect specific pathogens: different serological procedures to reveal seed-borne viruses and bacteria, the phage-plaque test for detecting seed-borne bacteria, and the indicator test for detecting both of these categories of pathogens as well as fungi.

Procedural details:

1. The Blotter or Filter Paper Test

The method is widely used to detect a number of fungi. The procedural details of this method are as follows (adopted from International Seed Testing Association):

a. A working sample of 400 seeds is tested in replicates of 25 seeds per dish of 9 cm diam. Since the

seeds are later incubated in light, the dishes should be of such a material that allows light to pass through, e.g. Petri dishes made from clear plastic, and glass dishes of Pyrex or Corning. In plant quarantine, the samples of seeds may be less than 400 . In such cases the whole sample or part of it may be tested. Only healthy seedlings, free from infection, must be released and grown in the quarantine glasshouse for inspection by the quarantine officer.

b. Label each dish properly with the accession number of the seed sample, date of examination and the dish number.

c. Before plating the seeds in the Petri dishes, each dish should be lined with three filter papers (blotters), well soaked in water. The water soaked filter papers for the dishes can be prepared in the following way: Count three filter papers at a time, dip them in water for a few seconds, lift and let the excess water drip off before setting the wet blotters in the dish.

d. Plate 25 seeds in each dish, 15 seeds in the outer ring, 9 in the middle and one in the centre.

e. Incubate the seeds at 20-22 C for 7 days in alternating cycles of 12 hr light and 12 hr darkness. Light should be supplied by two fluorescent tubes hanging horizontally, 20 cm apart from each other and the distance between the light tubes and the dishes should be 40 cm.

f. After 7 days incubation, start examination of seeds under a stereoscopic microscope with magnification at least up to x 50 or x 60. All seeds of the outer ring must be examined first, then the seeds of the second ring and finally the seed in the centre of the dish. Examination of seeds in sequence becomes easier when a line is drawn with a colored pencil. Examine thoroughly the whole seed at different magnifications before proceeding to the next seed.

Once the examination of the first seed is finished rotate the dish gently clockwise with the middle finger and the thumb of the left hand while still looking into the microscope. Follow this procedure in moving from one seed to the other.

g. Whenever the growth of an organism of interest is seen, mark the seed infected by writing near to the infected seed.

h. Identification of a particular organism needs experience in seed health testing. Whenever a growth is suspected of a particular organism, mount conidia (spores) in water on a glass slide and confirm the conidial morphology of a particular fungus under higher magnifications of a compound microscope.

i. Count the total number of seeds infected by a particular organism in each dishand enter the figures in a recording sheet, and calculate per cent seed infection.

2. The Agar Plate Test

Seeds are plated on an appropriate agar medium and spaced according to the size of the seed. Malt extract agar, oatmeal agar, or potato dextrose agar are most commonly used (see media preparations in Appendix (2).

Pretreatment of the seeds (soaking for 5-10 minutes in 1 per cent sodium hypochlorite) is applied to exclude fast growing saprophytes associated with the seeds. The following procedure is a more rigorous seed pretreatment. **a.** Put seeds in a screw capped tube containing about 25 ml of 30% (v/v) bleach (ie., 5% hypochlorite) 1 ul/ml of 20% Triton-X100, **b.** Shake vigorously so that a layer of foam develops on the solution. Most of the trash (which contains most of the contaminants) will collect in the foam which should be removed by aspiration with a sterile pasteur pipet, **c.** Continue to mix slowly by inversion for 5 to 10 minutes, **d.** Allow the seeds to settle for 1 or 2 minutes and decant the bleach, **e.** Fill the tube with sterile water, let seeds settle and decant, **f.** Repeat this process several times to remove as much bleach as possible. The seeds should be plated within a day. The incubation conditions are the same as described

under the blotter test.

3. Adult Plant Inspection

Certain seed-borne diseases require a longer period of incubation to be detected than is provided by the usual incubation procedure. The seeds are sown in autoclaved soil in suitable containers and submitted to optimum conditions of temperature and humidity protected against contamination.

iii. Summary on seed health testing

Detection of fungi: procedures using the blotter or filter paper method and agar media are usually sufficient. The agar media most commonly used are potato dextrose agar (PDA), malt extract agar, and oat meal agar.

Bacteria: procedures using appropriate culture media are adequate, but specific procedures such as phage-plaque or serological tests (provided that antisera are available) may be used. Nutrient agar and King's medium B are widely used.

Viruses: indicator tests (provided that suitable indicator plants are available), and ELISA can be used.

V. GENERAL PROCEDURES AND RECOMMENDATIONS

All agricultural scientists should understand that plant quarantine is a measure of plant disease control which we all want to achieve. It is our responsibility to take all the necessary precautions to prevent man-assisted pathogen disseminations.

i. General procedures for germplasm introductions.

The following procedures are representative of those followed by most quarantine officers in regulating germplasm of high risk genera.

1. Seeds should be collected from "disease-free" plants.

2. Small, discolored, shrivelled or damaged seeds should be avoided.

3. Seeds should be free of any plant residues such as leaf pieces, roots, glumes, husks or other foreign materials such as stones, soil particles, etc.

4. Seeds should not be packed using plant materials such as straws. All packing materials should be clean.

5. Seeds should be introduced, rather than vegetative materials, unless clonal propagation is a requirement or the plants are collected in the wild when seed is not available.

6. For clonal propagations, unrooted vegetative materials such as scions or unrooted cuttings are preferred to rooted plants.

7. Woody plant introductions should not be more than 2 years old.

8. Consignments of vegetatively propagated clonal germplasm should be small, i.e., limited to a few tubers, cuttings, or scions. If an accession is represented by tubers, cuttings, and scions, each

component should be regarded as separate subclone, particularly for virus indexing.

9. Germplasm that must be introduced as plants should enter free of soil. The original importation should be destroyed once daughter plants have been established.

10. If phytosanitary procedures require that clones, or subclones, be indexed for viruses, only propagations derived from indexed mother plants should be released.

11. With genera that present a high risk of seed-borne organisms, the original seed should be treated and planted to produce a healthy lot of seed.

12. For high-risk genera, whether imported as seed or vegetative material, only the part of the shipment that is passed through quarantine should be released. The part of the original introduction not used as mother or seed plants should be destroyed.

ii. Procedures for adult plant inspections in the glasshouse.

In exchanging genetic resources, the quantities of seed for distribution, and hence for sampling and testing, is very limited.

It seems more appropriate and realistic if all original seed introductions are examined at the Seed Health Lab. (currently under GRU) first, using appropriate seed health testing protocols, and those seeds which show no pathogens be planted in the plant quarantine green house for inspection. Visual plant inspection alone may not be effective and may provide only a false feeling of security.

The following steps should be taken in the phytosanitary glasshouse:

1. Pots, soil, and benches should be sterilized before being placed in the glasshouse.

2. The floor of the entrance cubicle should be treated with a disinfestant.

3. All planting of imported material should be carried out only in the glasshouse.

4. Personnel working in the glasshoue should be careful not to brush against plants or handle any plant unless necessary.

5. Hands and instruments should be washed with soap and water or some disinfestant after trimming, digging, etc., before being moved from one place to another.

6. Distance between potted plants should be sufficient to prevent contact with each other.

7. Airflow within the glasshouse unit should be controlled so that it does not cause the plants to touch each other.

8. All materials have to be inspected on individual plant basis.

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Appendix 1 : DEFINITIONS

1. **Seed health:** According to the definition of the International Seed Testing Association (ISTA, 1985), health of seed refers primarily to the presence or absence of disease-causing organisms such as fungi, bacteria and viruses, and animal pests.

2. **Plant quarantine:** It is a preventive measure against the introduction of pests and pathogens harmful to agriculture. It is basically the use of exclusion as a control strategy and is applied to both imported and exported materials.

3. **Incubation:** Seed maintenance in a condition favorable to pathogen growth or disease symptom expression.

Appendix 2 : CULTURE MEDIA RECIPES

Potato dextrose agar

Potato dextrose agar (Difco 0013)	39.0 g
Distilled water	1000 ml
or	
Potatoes, infusion from	200.0 g
Bacto-Dextrose	20.0 g
Bacto-agar	15.0 g
Distilled water to	1000 ml

Malt extract agar

Maltose	12.75 g
Dextrin	2.75 g
Glycerol	2.35 g
Bacto-peptone	0.78 g
Bacto-agar	15.0 g
Distilled water	1000 ml
or	
Malt extract	20.0 g
Peptone	5.0 g
Agar	15.0 g
Distilled water	1000 ml
or	
Malt extract agar (Difco 0112)	33.6 g
Distilled water	1000 ml

Oatmeal agar

Agar	5.0 g
Distilled water	500.0 ml

Melt.

Instant oatmeal (for babies)	40.0 g
Distilled water (cold)	250.0 ml

Mix. Combine mixed oatmeal with melted agar

Nutrient agar

Peptone	5.0 g
Beef extract	3.0 g
Agar	15.0 g
Distilled water	1000 ml

King's medium B

Proteose peptone	20.0 g
Glycerol	15.0 g
K_2HPO_4	1.5 g
$M_gSO_4.17H_2O$	1.5 g
Agar	15.0 g
Distilled water	1000 ml

Adjust pH to 7.2 before autoclaving the medium

Notes: Media should be sterilized the same day that they are prepared. Sterilization is completed in 20-25 min. at a temperature of 121 C.

Appendix 3: ADDRESSES OF PLANT QUARANTINE OFFICES[1]

Afghanistan
Director
Plant Protection and Quarantine
Ministry of Agriculture
Kabul

Albania
Bureau of Agriculture
Tirana

Algeria
Chef
Service de la Protection des Végétaux
Ministère de l'Agriculture
12, Boulevard Colonel
Amirouche
Algiers

American Samoa
Director
Agricultural Quarantine Services
Department of Agriculture
Pago Pago, 96799

Antigua
Director
Ministry of Agriculture
Lands and Fisheries
St. John's
West Indies

Argentina
Director General
Servicio Nacional de Sanidad Vegetal
Ministerio de Agricultura
Paseo Colon 922, 1o Piso
Oficina No 196
Buenos Aires

Australia
Assistant Director General
Plant Quarantine
Department of Health
Canberra A.C.T.

Austria
Director
Bundesanstalt für Pflanzenschutz
Trunnerstrasse 5
1021 Wien

Bahamas
Director of Agriculture
Ministry of Agriculture and Fisheries
P.O. Box 28
Nassau

Bahrain
Department of Agriculture
Plant Protection Section
P.O. Box 251
Manama

Bangladesh
Department of Agriculture
(Extension and Management)
Director of Agriculture (E&M)
Ansari Building
14/2 Topkhana Road, Dhaka-2

Barbados
Head
Division of Entomology and Quarantine
Ministry of Agriculture,
Science and Technology
P.O. Box 505
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Belgium
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Ministère de l'Agriculture
36 Rue de Stassart
1050 Bruselles

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Director of Agriculture
Belmopan

Benin
Service du Dévelopment
Région Agricole du Sud
B.P. 648
Cotonou

Bermuda
Plant Pathologist
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---

1 *Addresses taken from Plant Production and Protection Division publications of the FAO (1981) and from Export Summaries published by the USDA, Animal and Plant Health Inspection Service, Plant Protection and Quarantine Programs.*

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Campesinos y Agropecuarios
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Department of Agricultura Research
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Brazil
Director
Secretaria de Defensa Sanitaria Vegetal
Ministerio da Agricultura
Esplanada dos Ministerios
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Brunei
The Director of Agriculture
Department of Agriculture
Bandar Seri Begawan

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Director of Plant Protection
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BD. Christo Botev 55
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Burkina Faso
Le Directeur
Section de Lutte Antiacridienne
de Protection des Plantes et des Cultures
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Burma
The Director
Department of Agriculture
Ministry of Agriculture and Forests
Rangoon

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Chef
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du Burundi (ISABU)
Groupe de Phytopathologie
et d'Entomologie
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Bujumbura

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Service de la Protection des Végétaux
Ministère de l'Agriculture
Yaound

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Agriculture Canada
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Direcção Nacional de Agricultura
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Cayman Islands
The Director
Department of Agriculture
Grand Cayman

Central Afrinca Republic
Le Directeur
Direction de l'Agriculture
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N'Djamena

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Servicio Agrícola y Ganadero (SAG)
Ministerio de Agricultura
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Instituto Colombiano Agropecuario (ICA)
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Ministerio de Agricultura y Ganadería
San José

Côte d'Ivoire
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Service de la Protection des Végétaux
Ministère de l'Agriculture
B.P. V7
Abidjan

Cuba
Director
Dirección Nacional de Sanidad Vegetal
INRA
Departamento de Cuarentena Vegetal
La Habana

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Plantentuin Cascora
Willemstad

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Plant Protection Section
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Ministry of Agriculture
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Plant Protection Service
Ministry for Agriculture and Food
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Roseau

Dominican Republic
Dirección General de Foresta
Central de los Heroes
Santo Domingo

Ecuador
Jefe
Departamento de Sanidad Vegetal
Ministerio de Agricultura y Ganadería
Quito

Egypt
Ministry of Agriculture
Dokki
Cairo

Plant Quarantine Administration
Customs Gate 6
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El Salvador
Jefe
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Ministerio de Agricultura y Ganadería
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Ethiopia
Director
Department of Plant Production
and Protection
Plant Quarantine Service
Ministry of Agriculture and Settlement
Addis Ababa

Federal Republic of Germany
Federal Ministry of Food,
Agriculture and Forestry
Plant Protection Division
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Fiji
Director of Agriculture
Department of Agriculture
Rodwell Road
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Plant Quarantine Unit
Agricultural Research Centre
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Vantaa 30

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Direction de la Qualité
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Chef
Service de la Protection des Végétaux
Boite Postale 241
Fort de France
Martinique

Gabon
Direction des Services Agricoles
B.P. 43
Libreville

Gambia
Crop Protection Unit
Department of Agriculture
Cape St. Mary

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Staatl. Pfl.sch. u.
Pfl.quarantaenedienst
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PMB 5672
Moor Plantation
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General Province of Agriculture
Ministry of Agriculture, Fisheries,
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Department of Plant Protection
Ministry of Agriculture and
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Seccion de Cuarentena Agropecuaria
Ministerio de Agricultura
Comercio e Industrias
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Stock and Fisheries
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Ministerio de Agricultura y
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Ministerio de Alimentación
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Ministerio de Trabajo
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Bureau of Plant Industry (BPI)
692 San Andres
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(Ministry of Agriculture)
Department Produkcji Roslinnej
i Ochrony Roslin
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Repartiçao de Serviços
Fitopatologicos (R.S.F)
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Bureau de la Protection Végétale
Ministère de l'Agriculture
et de l'Elevage
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Director
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Ministry of Agriculture and Water
Riyadh

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Direction de la Protection des Végétaux
Ministère du Developpement
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Director of Agriculture
Victoria
Mahe

Sierra Leone
Director of Agriculture
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Sri Lanka
Director of Agriculture
Department of Agriculture
Ministry of Agricultural
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Ministry of Agriculture and Lands
Castries

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Monsieur le Chef du Service
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Lands and Fisheries
Centeno

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Department of Resources
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General Directorate of
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Tuvalu (Gilbert and Ellice Islands)
Ministry of Commerce and
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Funafuti

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Animal and Plant health
Inspection Service
Plant Protection Service
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Dirección de Sanidad Vegetal
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Chef
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Port Villa

Venezuela
Director
Ministerio de Agricultura y Cría
Departamento de Sanidad Vegetal
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El Conde
Caracas

Vietnam
Plant Protection Service
Department of Rural Affairs
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Virgin Islands
The Director
Department of Agriculture
Road Town
Tortola

Western Samoa
Director of Agriculture
Department of Agriculture and Forests
P.O. Box 206
Apia

Yemen Arab Republic
Plant Protection Division
Ministry of Agriculture
Sana'a

Yemen, People's Democratic Republic of
Plant Protection Project
El Ked Agricultural Research Centre
Ministry of Agriculture
Aden

Yugoslavia
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Department for Plant Protection
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