

Plant health and germplasm collectors

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Introduction: the need for healthy germplasm

The movement of plant germplasm can potentially spread pests, defined in the *International Plant Protection Convention* (IPPC) as encompassing all harmful or potentially harmful biotic agents from viroids to weeds (in some cases, the plant species collected is itself actually or potentially a pest). In recognition of this danger, most countries have legislation to regulate the entry (and sometimes the internal movement) of plants, plant parts and their products. Consignments of germplasm infested with pests or of plant species considered pests and material without proper documentation may be refused entry and destroyed or re-consigned. Collectors of plant germplasm need to be aware of these facts, or they may fail to accomplish their goals. Germplasm should always be collected, processed and shipped with the necessary phytosanitary precautions to avoid accidental transfer of pests.

There are other, perhaps less obvious, reasons why pests should be given attention when germplasm is collected. Pests may affect the quality, and therefore the usefulness, of germplasm samples. Infection by pathogens can reduce the viability of seeds during storage. When material is multiplied, growth may be distorted, colours altered and disease susceptibility increased. These changes may make it difficult, if not impossible, to collect characterization and preliminary evaluation data, and some important characteristics, crucial for plant improvement schemes, may go undetected. In addition, infested samples are unlikely to be distributed. They cannot be grown out and regenerated and if stored they will remain unused and deteriorate.

It is therefore important to know what pests are likely to be associated with the target gene pool. This will allow an assessment of the risks associated with moving the germplasm and for appropriate

measures to be devised to reduce the risk to a minimum. It is also important to document the pests present on the target species at the time of collecting. This information, part of the passport data of the sample, will improve the usefulness of the germplasm and will also help during quarantine examination.

For all these reasons, it will often be useful to include a plant protection specialist in collecting teams if funds and logistical considerations allow. Preferably, this should be a plant pathologist experienced in the species to be collected, as pathogens are more difficult than insects and mites to detect during collecting and to eradicate from plant samples. If a plant pathologist cannot participate in the collecting mission, collectors should become familiar with the major pests of the target species. In all cases, collectors will have to ensure that the proper documentation has been assembled so that plant samples reach their intended destination unhindered.

This chapter gives guidelines on how these issues may be addressed. It considers what must be done at the planning stage, while collecting in the field and, finally, just before samples are dispatched.

Planning the collecting mission

At the planning stage, attention must be given to the pests that might be encountered on the target species and to the regulations governing plant movement. The following questions need to be considered when assembling information on plant pests:

- What pests have been recorded on the target species in the country of collecting, especially in the target area?
- What plant parts are they found on?
- How are the pests transmitted?

The following questions need to be answered to ensure compliance with phytosanitary regulations:

- What is the final destination(s) of all subsamples?
- What are the phytosanitary import requirements of the country(ies) of destination?
- What are the procedures for obtaining a phytosanitary certificate in the country of collecting?

Assembling information on pests

Pest surveys should be consulted to determine which pests have been recorded on the target species in the collecting region. However, in many countries such surveys are far from complete. Sometimes, they have not been done at all, are outdated or do not cover the entire country, concentrating on the more easily accessible areas. Another problem is that surveys mostly record pests of crop plants, neglecting wild relatives.

This lack of information is a major barrier to the formulation of quarantine regulations appropriate to the exchange of germplasm of some crops.

Texts that may be consulted for information on the pests of specific crops are listed under References. Holliday (1989) includes a list of major texts on plant pathology, including crop-specific disease compendia and references to these under crop names. On pest distributions, the following are important sources:

<i>Distribution Maps of Pests</i>	IIE (1968 <i>et seqq.</i>)
<i>Distribution Maps of Plant Diseases</i>	IMI (1942 <i>et seqq.</i>)

Collectors should confirm with the relevant institutes that these maps contain the most up-to-date information. Detailed descriptions, including notes on the transmission of many of the pests figured in the maps, can be sought from the following publications:

<i>Descriptions of Fungi and Bacteria</i>	IMI (1964 <i>et seqq.</i>)
<i>Descriptions of Plant-parasitic Nematodes</i>	IIP (1972 <i>et seqq.</i>)
<i>Descriptions of Plant Viruses</i>	CMI/AAB (1970-1984), AAB (1985 <i>et seqq.</i>)

On viruses, CAB International (CABI) and the Australian National University have collaborated on a major database, Virus Identification Data Exchange. *Viruses of Tropical Plants* (Brunt *et al.*, 1990) is an output of the database, and the publication of a further comprehensive book (*Viruses of Plants*) is planned by CABI in 1995. The commodity international agricultural research centres (IARCs) also publish useful illustrated guides to the pests of their mandate crops. These are particularly useful in the field.

In addition, a series of booklets of crop-specific *Technical Guidelines for the Safe Movement of Germplasm* is published jointly by the Food and Agriculture Organization (FAO) and the International Plant Genetic Resources Institute (IPGRI) (formerly IBPGR). Each booklet is divided into two parts. The first part makes recommendations on how best to move germplasm of the crop concerned and lists institutions recovering and/or maintaining healthy germplasm. The second part covers the important pests and diseases of quarantine concern, giving a description of therapy and indexing methodologies. So far, guidelines have been produced for the following crops:

aroids (edible)	Zettler <i>et al.</i> (1989)
<i>Citrus</i> spp.	Frison and Taher (1991)
<i>Cocos nucifera</i>	Frison <i>et al.</i> (1993)
<i>Dioscorea</i> spp.	Brunt <i>et al.</i> (1989)
<i>Ipomoea batatas</i>	Moyer <i>et al.</i> (1989)
legumes	Frison <i>et al.</i> (1990)
<i>Manihot esculenta</i>	Frison and Feliu (1991)
<i>Musa</i> spp.	Frison and Putter (1989)

<i>Saccharum officinarum</i>	Frison and Putter (1993)
<i>Theobroma cacao</i>	Frison and Feliu (1989)
<i>Vanilla</i> spp.	Pearson <i>et al.</i> (1991)
<i>Vitis vinifera</i>	Frison and Ikin (1991)

Guidelines for small fruits (*Fragaria*, *Ribes*, *Rubus* and *Vaccinium*) were published in 1994, and booklets on *Allium*, *Brassica*, *Oryza*, *Solanum* potatoes, *Zea* and forestry trees are planned. Hewitt and Chiarappa (1977) is an earlier, crop-by-crop analysis of the problems and risks attendant on the transfer of plant germplasm.

The Plant Protection Service of FAO, in collaboration with regional plant protection organizations, has developed a database of plant pests worldwide, which is updated and distributed regularly to member countries. This Global Plant Quarantine Information System describes the geographic distribution of each pest, specifies hosts and commodity type affected, comments on quarantine status and describes preferred treatments. Lists of pests affecting particular hosts may be generated. For each country, the database provides lists of pest records, digests of plant quarantine regulations and information on the national plant quarantine organization. Based on the information in the database, the software can assess the quarantine risk associated with particular consignments. Updating of the database is coordinated with the regional plant protection organizations, CABI and other research institutes involved in verification of pest distribution and damage reports.

Assembling the required plant health documents

It is essential to begin making phytosanitary arrangements as soon as possible. Delays in obtaining the appropriate documents are common, but without these documents the mission may be postponed or, worse, the samples destroyed. It is the responsibility of collectors to obtain the necessary documents in order to transfer plant germplasm. Two documents are commonly required for international transfer: an import permit and a phytosanitary certificate.

The import permit

The import permit is obtained from the country or countries of destination of the germplasm before the mission sets out. At this stage, information is also gathered on how to obtain a phytosanitary certificate in the country of collecting and whether other authorizations are required to export germplasm. The FAO/IPPC Secretariat has a list of plant protection services worldwide with contact addresses of the authorities responsible for issuing these documents. This information is also available in the Global Plant Quarantine Information System and as a hard-copy directory (FAO, 1993).

An import permit, issued by the quarantine authorities of the importing country, stipulates the conditions governing plant introductions. If

samples are to be collected and sent to more than one country for safety duplication, study or use, each country has to be approached to supply an import permit.

No general rules apply. Regulations differ among countries according to the perceived risks involved in making the importation. There are a number of different possibilities and the conditions of entry will be detailed on the import permit. Even when no conditions apply and germplasm is allowed unconditional entry, a document from the plant protection service of the importing country to that effect should be obtained.

Collectors should ensure that the number of samples and the approximate size of each are known to the importing country well ahead of arrival, so that the quarantine inspection service can properly plan to process the samples. This is particularly important if samples are to be grown in post-entry quarantine in the importing country or in a third country.

Usually, two copies of the import permit are obtained. The top copy should always accompany the consignment. A photocopy is usually allowed for multiple consignments. A copy of the import permit must be retained by the collector.

The phytosanitary certificate

The phytosanitary certificate is issued by the quarantine authority of the exporting country, certifying that the product meets the phytosanitary regulations of the importing country. Consignments are inspected and the certificate issued if they are 'free from quarantine pests and practically free of injurious pests' (see the IPPC model phytosanitary certificate, Appendix 17.1 at the end of this chapter). A 'quarantine pest' is different from a merely 'injurious pest' in this jargon in that it is of potential national economic importance to the country and not yet present there, or present but not widely distributed and being actively controlled.

In some instances, in order to reduce the overall pest risk, germplasm consignments will need to be given phytosanitary treatments in the country of origin (but see Chapter 20 on the potential risks for seed viability of such treatments). Fumigation may be requested or the samples may be dipped or dusted in an insecticide or fungicide, given a hot-water treatment, or whatever is considered appropriate by the importing country. The treatments should be applied exactly as requested. The permit may seek additional endorsements. These, as well as the treatments, should be detailed on the phytosanitary certificate. Finally, the certificate should be signed by the duly authorized government representative.

Under no circumstances should treatments be applied as alternatives to those of the import permit without first requesting the authority of the importing country. Likewise, if no treatments are

requested, none should be given, since importing countries may wish to inspect or test germplasm consignments, and treatments already applied to seeds may mask symptoms of seed-borne pathogens and interfere with laboratory tests. Alternatively, treatments already applied may be ignored, in which case a second treatment could reduce viability. If seeds are pretreated prior to entry, against the conditions of the permit, this could seriously jeopardize their importation.

Where germplasm samples are to be sent to more than one country, it is necessary to obtain a phytosanitary certificate for each destination. It is important that the certificate(s) should be issued without amendment or erasure. Many countries refuse to accept altered certificates. A fee may be charged for fumigation or disinfection treatments and, occasionally, for inspection.

Two copies of the phytosanitary certificate should be obtained. The original should accompany the consignment.

Documentation and intermediate quarantine

Collectors are also responsible for arranging the documentation for germplasm samples that have to be grown in intermediate (third-country) quarantine. Such arrangements are necessary when it is unsafe to make transfers directly to the importing country, but quarantine multiplication may be necessary even if the material is not to leave the country of collecting. Procedures are essentially similar to those outlined above. An import permit must be obtained from the quarantine authority of the intermediate country. A copy of this must accompany the consignment, together with a phytosanitary certificate showing any treatments or endorsements requested on the permit. After the samples have been grown in intermediate quarantine and declared safe for further transfer, an import permit must be obtained from the country of final destination and a new phytosanitary certificate issued by the intermediate country.

Planning the identification of pests

Misidentifications of pests can seriously jeopardize the usefulness of consignments. Identification services for fungi, bacteria, nematodes, insects and mites are provided by CABI. Costs vary depending on whether or not a country is a member of CABI. CABI also publishes useful directories of organizations, for example Hall and Hawksworth (1990). It may be possible to arrange for the identification of important seed-borne diseases of tropical countries by the Danish Government Institute of Seed Pathology for Developing Countries, Hellerup, Denmark. Identification of virus and virus-like infections is more problematical. Specimens will need to be sent to institutes specializing in particular crop plants. Lists of institutes providing this service can be found in the appropriate booklet in the FAO/IPGRI series of safe transfer guidelines. The Tropical Virus Unit at the Institute of Arable Crops Research, Rothamstead Experimental Station, UK, is an example.

In all cases, arrangements must be made well ahead of dispatch to allow the orderly processing of specimens. Import permits may be needed. If so, these must be obtained from the appropriate authorities in the country where specimens are to be examined. Collectors should ensure that the institutes making the identifications know where to send the results.

In the field

Minimizing the pest risk

Familiarity with the symptoms caused by pests and with which plant parts are most likely to be contaminated by the different pests of concern is essential. In general, the risk of spreading pests with germplasm is greatest if rooted plants are moved. This is because of the likelihood that nematodes and other soil-borne pathogens will be present: these are difficult to treat without destroying the plant tissues. Other types of vegetative propagating material (e.g. stems, bulbs, corms, etc.) also present a risk, mainly because of infection from systemic pathogens. The international movement of seeds and pollen is considered safer, as fewer pests are harboured by these plant organs. Phytosanitary considerations may therefore contribute to the decision as to what plant part(s) to collect.

It may be possible to apply curative treatments to lessen or eradicate the pest risk. For surface-borne pathogens and insects, pesticide treatments and fumigation may be tried. Where virus, virus-like organisms and internally borne fungi and bacteria are a threat, thermotherapy and shoot-tip culture are most appropriate.

For vegetatively propagated species, transfer of germplasm as *in vitro* cultures will greatly reduce the pest risk. Nevertheless, it should be stressed that *in vitro* culture *per se* does not eliminate the risk entirely. It should be complemented by indexing (testing) for viruses and virus-like organisms that are suspected to be present in the area where the germplasm was collected.

The safe transfer guidelines give general advice to collectors on the type of germplasm considered safe to move internationally, and detailed technical recommendations on how the germplasm may be treated to ensure that it is free of pests. In some instances, because of the severity of the pest and the difficulty of collecting healthy material from the field, the guidelines advise transfer of material through a third country, where therapy and indexing procedures can be carried out to ensure freedom from internally borne pathogens. The general recommendations of the guidelines are useful even for crops not specifically covered in the series to date.

Recording data on pests

It is important for collectors to record the pests present on their target species and whether other pests are present in the target region. Noting that plants are free of pests in an area where pests are common is equally important. Collectors should attempt to describe the symptoms caused by pests. It is, however, often difficult for someone untrained in plant pathology or entomology to do this. Symptoms may be caused by a combination of several pests, or the causal agent may be obscured by the presence of a minor one or by an opportunistic saprophyte. Symptoms due to root attack or internal pathogens are often particularly difficult to interpret. Where there is doubt as to the identification of pests, plant specimens showing typical symptoms should be collected and dried or preserved by other means, as appropriate (see below).

A description of symptoms should include information on the following (Sonoda, 1979):

- the general condition of the plant;
- the plant part(s) affected;
- the type of damage;
- the stage of growth affected.

Rating the severity of attack, both in terms of its effect on the individual plant(s) affected and in terms of the percentage of the population affected, will increase the value of the information. Descriptor lists are published by IPGRI for many crops, and these catalogue the important pests and give scales of severity. Colour photographs showing the full range of symptoms, including close-ups of damaged areas and of the pests themselves, are often useful diagnostic tools (Sonoda, 1979).

Farmers' knowledge of pests can be extensive and detailed. Some examples are given by Altieri (1993). Collectors can often complement the kinds of observations described above with discussions with knowledgeable local people.

Preservation of pests associated with germplasm samples

Correct identification of pests depends on the quality of the specimens prepared in the field. Collectors should be equipped at least with specimen bottles, alcohol (75% isopropyl alcohol) and formalin for preserving insects, mites and nematodes, and with newspapers and plant presses for making dried herbarium specimens of plants with fungal and bacterial diseases (Chapter 27). Specimens may need to be shared among several institutes, and sufficient material should be collected to allow this.

Sonoda (1979) gives guidelines on capturing, killing and storing insects and other pests in the context of germplasm collecting. For insect pests, representative specimens of all life stages may be necessary for taxonomic identification. Insects can be captured using nets, by beating plants over a cloth or by using an aspirator. They can be killed using potassium cyanide or ethyl acetate, both of which are dangerous

and should be clearly labelled and properly stored. Some must be pinned (e.g. Diptera and Hymenoptera), others can be stored in alcohol (e.g. beetles) and others can be stored in small envelopes (e.g. Lepidoptera).

Dried specimens of diseased plants should include as much of the plant as possible, showing both old and new lesions if possible. Fresh specimens can also be collected and stored in plastic bags. They will remain useful longer if refrigerated. Fungal and bacterial pathogens may be isolated from diseased plants in the field, but this requires sterile technique and will not often be feasible in the context of plant germplasm collecting.

Plants infected with viruses or virus-like organisms present the collector with the greatest challenge, as the material needs to be processed in different ways according to the type of pathogen. Where tissues are thought to contain non-cultivable mollicutes (formerly referred to as mycoplasma-like organisms), they need to be fixed in glutaraldehyde, whereas tissues for virus examination may be sent fresh, dried as thin sections over calcium chloride or as sap stained on electron-microscope grids. Because of the complexity of the subject, it is essential that prior to departure collectors seek advice on the preservation of specimens from the institutes where the specimens are to be sent for examination.

Details of methods of preserving various kinds of diseased material can be found in *The Plant Pathologist's Pocketbook* (Johnston and Booth, 1983). Methods for collecting and preserving different insect groups can be found in Bland and Jacques (1978), British Museum (Natural History) (1974) and Borror *et al.* (1976).

Back at base: treatment and dispatch of germplasm samples

This section gives a summary of the phytosanitary procedures involved in handling plant germplasm after it has been collected and brief notes on the dispatch of specimens for pest identification. For other aspects of the tasks that will need to be undertaken once back at base, see Chapter 28.

Inspection

Missions should carefully prepare germplasm samples before they are presented to quarantine authorities for inspection, treatment and certification.

- Germplasm samples should be carefully inspected for pests, insects and mites as well as for lesions or colour patterns which may denote fungal, bacterial or viral pathogens. Where such pests, or symptoms of pests are present, the pests and/or the symptom-bearing seeds should be removed.
- Bare-rooted plants should be thoroughly washed to ensure they are

free of soil, which might harbour nematodes and other soil-borne pathogens.

- Seeds and pollen should be free of debris. If this is present, it should be removed.

Phytosanitary treatments and certification

- If mandatory treatments are prescribed on the import permit or endorsements are required, these should be given by the relevant government authority exactly as requested.
- After treatments have been applied, they should be detailed on the phytosanitary certificate, together with any other endorsements, as requested by the importing country.
- Two copies of the phytosanitary certificate should be obtained, one of which should be the original. Each copy should bear the stamp of the organization issuing the certificate, and be signed by an authorized officer.
- Collectors should ensure that the phytosanitary certificate contains the following information:
 - name and address of the exporter;
 - name and address of the consignee;
 - number of samples of each species in the consignment;
 - botanical name of each species;
 - phytosanitary treatments applied;
 - additional endorsements required by the import permit.

Documents accompanying germplasm consignments

- The original copy of the phytosanitary certificate, plus a copy of the import permit, should accompany the consignment. Photocopies may be allowed if there are multiple shipments. This will have to be ascertained from the quarantine authorities of the importing country. The top copy of the permit should be placed on the outside of the package so it can be forwarded to the plant quarantine authorities without the need to open the package. A photocopy should be included inside the package in case of damage to the outside copy. However, this may vary from country to country. For example, US regulations specify that all documents should be inside the package.
- A copy of all documents sent with the consignments should be retained by the collector.

Preparation of samples for pest identification

Arrangements should be made in advance of the fieldwork with the institutes that are to receive samples for pest identification. Permits may have to be obtained to comply with the quarantine requirements of the country where samples are to be sent. Some additional points to note are:

- All material sent for identification purposes, whether preserved insects and mites, dried plant voucher specimens of diseased plants or living plant material for diagnosis of internal pathogens, should be labelled with:
 - a reference number;
 - the botanical name of the host plant;
 - the locality where collected;
 - the date of collecting;
 - the name of the collector(s).
- Collectors should keep a copy of the information accompanying each specimen.
- Samples of seeds and pollen may have to be sent for viability testing, as well as for inspection for internally borne pathogens and weeds. Samples should be properly dried before dispatch.
- Collectors should include the name of the person (and address) to whom the identification(s) should be sent.

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APPENDIX 17.1

IPPC model phytosanitary certificate

No. _____

Plant Protection Organization of _____

To: Plant Protection Organization(s) of _____

Description of consignment

Name and address of exporter _____

Declared name and address of consignee _____

Number and description of packages _____

Distinguishing marks _____

Place of origin _____

Declared means of conveyance _____

Declared point of entry _____

Name of produce and quantity declared _____

Botanical name of plants _____

This is to certify that the plants or plant products described above have been inspected according to appropriate procedures and are considered to be free from quarantine pests, and practically free from other injurious pests; and that they are considered to conform with the current phytosanitary regulations of the importing country.

Disinfestation and/or Disinfection Treatment

Date _____ Treatment _____ Chemical (active ingredient) _____

Duration and temperature _____ Concentration _____

Additional information _____

Place of issue _____

(Stamp of organization) Name of authorized officer _____

Date _____

(Signature)

No financial liability with respect to this certificate shall attach to _____

(name of Plant Protection Organization) or to any of its officers or representatives.*

*Optional clause