

**HANDBOOK  
FOR THE**

**Collection, Preservation and  
Characterization  
of Tropical Forage  
Germplasm Resources**

CIAT is a nonprofit organization devoted to the agricultural and economic development of the lowland tropics. The Government of Colombia provides support as host country for CIAT and furnishes a 522-hectare farm near Cali for CIAT's headquarters. In addition, the Fundación para la Educación Superior (FES) makes available to CIAT the 184 hectare substation of Quilichao, situated near Santander de Quilichao, Departamento del Cauca. Collaborative work with the Instituto Colombiano Agropecuario (ICA) is carried out on several of its experimental stations and similar work is done with national agricultural agencies in other Latin American countries. CIAT is financed by a number of donors represented in the Consultative Group for International Agricultural Research (CGIAR). During 1979 these donors are: the United States Agency for International Development (USAID), the Rockefeller Foundation, the Ford Foundation, the W.K. Kellogg Foundation, the Canadian International Development Agency (CIDA), the International Bank for Reconstruction and Development (IBRD) through the International Development Association (IDA) the Inter-American Development Bank (IDB), the European Economic Community (EEC) and the governments of Australia, Belgium, the Federal Republic of Germany, Japan, the Netherlands, Norway, Switzerland and the United Kingdom. In addition, special project funds are supplied by various of the aforementioned entities plus the International Development Research Centre (IDRC) of Canada and the United Nations Development Programme (UNDP). Information and conclusions reported herein do not necessarily reflect the position of any of the aforementioned agencies, foundations or governments.

Series 05EG-1

June, 1979

**HANDBOOK  
FOR THE**

**Collection, Preservation and  
Characterization  
of Tropical Forage  
Germplasm Resources**

**Technical Editor:** G.O. Mott  
Agronomy Department  
University of Florida

**Production Editor:** Alejandro Jiménez C.  
Communication Support Unit  
CIAT

**Centro Internacional de Agricultura Tropical (CIAT)  
Apartado Aéreo 6713, Cali, Colombia, S.A.**

This publication is the result of a workshop on the Collection, Preservation and Characterization of Tropical Forage Germplasm Resources, held at the Centro Internacional de Agricultura Tropical (CIAT), on April 11-14, 1978. The workshop was co-sponsored by the Center for Tropical Agriculture, University of Florida; the U.S. Agency for International Development; and CIAT. The workshop and the preparation of the manuscript for this handbook were supported by Grant AID/ta-G-1425 from the U.S. Agency for International Development to the University of Florida.

## CONTENTS

Foreword :		vii
Introduction :	Strategies for the Collection and Improvement of Tropical Forages <i>G.O. Mott and E. Mark Hutton</i>	1
I :	Preparation for Collection Trip <i>R. Schultze-Kraft</i>	5
II :	Germplasm Collection in the Field <i>R. Schultze-Kraft</i>	9
III :	Description of the Collection Site <i>R. Reid and J.R. Lazier</i>	15
IV :	Soil Sample Collection and Procedures <i>L.A. León, W.E. Fenster, and P.A. Sánchez</i>	17
V :	Collection of Strains of <i>Rhizobium</i> <i>R.A. Date and J. Halliday</i>	21
VI :	Collection and Preservation of Insects and Pathogenic Organisms <i>R.M. Sonoda</i>	27
VII :	Characterization and Preliminary Evaluation <i>A.E. Kretschmer, Jr.</i>	33
VIII :	Transfer of Forage Germplasm <i>R.A. Luse</i>	45
IX :	Preservation of Forage Germplasm <i>R.A. Luse</i>	51
X :	Data Management of Forage Germplasm <i>L. Song and K. Rawal</i>	61
Contents		v

Appendix 1 :	List of descriptors for forage germplasm collection and characterization	65
Appendix 2 :	Dictionary of descriptors for forage germplasm collection and evaluation	71
Appendix 3 :	Procedure for sending nodule samples to CIAT	86
Appendix 4 :	List of laboratories which will isolate strains of <i>Rhizobium</i> on behalf of collector	86
Appendix 5 :	Sample form for seed export in India	88
Appendix 6 :	List of countries and the authorities issuing phytosanitary certificates	89
Appendix 7 :	Main recommendations and conclusions of the International Board for Plant Genetic Resources (IBPGR) Working Group on Engineering, Design and Cost of Long-term Seed Storage Facilities	91
Appendix 8 :	Recommended conditions for germination tests of forage legumes and grasses	93
Appendix 9 :	Alphabetic codes for countries	94

## FOREWORD

A workshop was organized to coordinate and plan for the Collection, Preservation, Distribution and Characterization of Germplasm Resources of Tropical Forages. This was the first major effort to bring together the forage scientists from several countries of the world to discuss means of coordinating their activities associated with the collection and characterization of forage germplasm resources.

The planning phases were conducted in cooperation with the three co-sponsoring institutions. The organizing committee was responsible for the selection of topics and authors of this handbook. Emphasis was given to invite outstanding scientists who are actively engaged in the various aspects of this activity.

The executive committee was responsible for the selection of the participants and of those who were invited to make oral presentations.

The International Center for Tropical Agriculture (CIAT) offered to host the workshop and provided its excellent facilities and logistical support. The U.S. Agency for International Development awarded the University of Florida a special grant AID/ta-G-1425 to cover the travel of some participants and to cover partially the publication costs.

Most of the workshop was devoted to informal discussions among the participants on the topics discussed in the handbook. During the deliberations the chapter authors were charged with the responsibility of acquiring participant response to the preliminary draft so that this handbook would represent a consensus of those participating.

The organizing and executive committees are especially grateful to the chapter authors for preparing preliminary drafts of their chapters which were distributed at the opening of the workshop, to the participants for their active and candid dialogue and to the authors for their efforts to prepare their chapters in final form.





# INTRODUCTION

## STRATEGIES OF THE COLLECTION AND IMPROVEMENT OF TROPICAL FORAGES

*G.O. Mott and E. Mark Hutton*

Plant evolution has generated the genetic variability and the wide diversity of genera, species, and ecotypes of tropical forages available today. It is imperative that we take full advantage of what has been learned by trial and error in other crops and what already has been done by various organizations in collection and evaluation of legumes and grasses. We need to apply all available scientific technology to the rapid development of new forage cultivars, which we believe will improve the quantity and quality of forage production.

It is vital to collect a greater range of native ecotypes of promising legumes and grasses as soon as possible before they are eliminated in many areas through clearing, cropping and land exploitation, stimulated by the rapidly increasing populations in many countries. At present, we probably have collected only a small fraction of the available genes of potentially valuable legumes and grasses.

The activity to which this workshop is dedicated is in its initial stages. We are just beginning the monumental task of trying to coordinate the collection, preservation, distribution, and characterization of a large number of species and ecotypes of both legumes and grasses. It will be very easy to accumulate an unmanageable mass of germplasm in a multitude of species, unless we take steps now to standardize procedures. The technology already available could be improved. It is up to us to find means of applying the best technology to this activity, so that each agricultural scientist may be able to retrieve the germplasm and information available wherever he may be working. We would like to emphasize that the time is now, before the number of collections exceeds the capacity of germplasm banks to cope.

It is important to remember that we are not only just collecting or generating information for ourselves, but also for the forage scientists in the tropical world.

## **Centers of Diversification**

With our great reservoir of genera and species of tropical forages, how important is it for us to identify centers of diversification? Will there be a tendency to concentrate our collecting activities in these centers when perhaps the genes we are really looking for are those which are on the perimeters?

Should we not be more concerned with clearly describing the environmental niches from which the collections are taken? Is it important for us to catalogue the forage legumes, particularly those in genera with the most promise, found in the major herbaria of the world? Should the cost-benefit ratios of such a procedure be considered in the development of new cultivars? Many herbarium sheets furnish only the botanical name and the collector. In contrast, other sheets give a very complete description of the collection site and one has the feeling he could go to the exact spot and collect the same ecotype. The organizing committee set as one of its objectives the preparation of distribution maps of the most important genera and species.

## **Ecophysiology of Tropical Forages**

The evolution of forage plants in the tropics has been a response primarily to natural forces and the migration of species into new environments where other stresses have been imposed and new recombinations of characters have occurred. Selection pressures have been those imposed by climate, moisture and mineral stresses, and the competition of other species in the environment which we might combine as the biotic factors. Only to a very small degree has man intervened in the forage selection process, and certainly not to the extent that he has intervened in the evolution and selection of his food crops.

In more recent times, man has introduced the domestic ungulate animal into the grassland ecosystem and many ecotypes of both grasses and legumes have perished under the stress of the grazing animal. In the selection process and in the development of new cultivars, persistence under grazing or cutting becomes very important and perhaps more important than yield potential. Since stress imposed by the grazing animal, especially at high grazing pressures, is frequently not a method of screening employed during the early phases of evaluation of new cultivars, the risk of failure under farm conditions is very high. This means that we must take a serious look at the screening procedures currently being used by many agronomists and plant breeders.

As research on tropical grasses and legumes progresses, one is impressed by the adaptations which have occurred between and within species with respect to

tolerance of moisture and mineral stress. The range of tolerance to acidity and deficiency of phosphorus, as in species of the genus *Stylosanthes*, is important. We need to search the germplasm spectrum for legumes which will tolerate the extremes of the mineral environments of tropical soils such as low P and Ca and low pH combined with high Al and Mn.

## **Legume-Rhizobium Relationships**

In the past two decades, the research on *Rhizobium* has been concentrated on host plant-*Rhizobium* specificity and the efficiency of nitrogen fixation. Unlike laboratory conditions, the host and the bacteria in tropical acid soils are subjected to scores of factors and interactions related to soil pH, Al and Mn toxicity, P deficiency, micronutrient deficiencies and excesses, and many others. Are selected rhizobia which are used in inoculation able to survive in such an environment against the competition from native rhizobia and other microorganisms? New methods for laboratory selection of rhizobia, particularly for acid tropical soils, are needed. However, we have to help the microbiologist in his search by collecting the *Rhizobium* along with the legume host plant. The legume and its associated *Rhizobium* should be considered an inseparable unit in the collection process.

## **Cultivars from Germplasm Resources**

The development of cultivars is not the exclusive job of the pasture agronomist or plant breeder. It includes a large spectrum of disciplines. Few will have the facilities, resources or personnel to cover as many aspects of evaluation as has CIAT, which is, of course, working for forage scientists in all tropical countries. What then can you do? Where do you enter this evaluation program? How can you best utilize the resources which you have that will provide the greatest probability of success of a new cultivar? These are difficult questions to answer. The more information generated during the collection process concerning the description of the collection site, the physical and chemical properties of the soil, and the insects, diseases, and *Rhizobium* associated with the collection, the better able you will be to select the accessions most likely to succeed in your environment.

## **Utilization of New Cultivars**

The researcher must never lose sight of the ultimate use of a new cultivar during the entire evaluation program. Will it be used by large ranchers, small farmers, in shifting agriculture? Will it be used for hay, cut forage, green manure, or for pasture? The use to be made of a new cultivar will determine, in a large

measure, the methods to employ in evaluation and the choice of the attributes for which you as a researcher will be looking.

The inclusion of a legume in farming systems has three primary purposes: (1) to add nitrogen through biological nitrogen fixation, (2) to serve as livestock feed, (3) and to provide green manure in a rotation of multiple cropping. Biological nitrogen fixation has been placed first since it is already well known that legumes can add up to 200 kg/ha of nitrogen each year to the system. In the grazing system the fixed nitrogen will supply animal requirements as forage protein, but a large portion of it needs to be retained in the soil-plant-animal recycling stream. The potential for increasing production of tropical grasslands, if we can learn to manipulate this system, is great indeed.

### **Storage, Preservation and Retrieval**

In the over-all strategy, storage, preservation, and retrieval are the most important and expensive components of this activity. It is not a problem to maintain a workable system for only a few hundred accessions, but when the numbers reach the tens of thousands or more we must adopt the latest technology available. With tropical forages we are fortunately at the stage when accumulation of collections in our germplasm banks is just beginning to escalate.

The product of the workshop is this handbook, which we hope will serve as a general guide to those engaged in one or more of the activities discussed.

# I

## PREPARATION FOR COLLECTION TRIP

*R. Schultze-Kraft*

Preparation for a collection trip can be divided into two phases:

1. Preparation with respect to information on the target and objective of the trip (region to be explored and germplasm to be collected).
2. Preparation with respect to the equipment necessary for germplasm collection.

In each case a distinction should be made between general and technical aspects of preparing for a trip.

As to general aspects, preparation for collecting forage plant germplasm does not differ from that of any other field trip for research or recreation purposes in possibly rather desolate regions. For this reason, emphasis will be placed mainly on that part of preparation related to the specific objectives of the collection.

### **Data on the Region to Be Explored and Germplasm to Be Collected**

#### **General information**

The most obvious general information that has to be gathered when planning a collection trip refers to the existence and accessibility of highways and roads, as well as the logistic support data. This data includes possibilities for spending the night, availability of potable water and edible food, availability of oil and gasoline for the vehicle, and need and availability of expert guides familiar with the region to be explored. It is very important to obtain this information from a trustworthy source; that is, directly from persons who really know the region.

## Technical information

Trip reports made by scientists who have visited the same region in the past, as well as data on vegetation, soils, topography and climate, will be very useful to the collector. This type of information will give him a better knowledge of the region and will simplify identification of priority zones. Data on germplasm collections previously made by other scientists also will be very valuable. In addition, it is advisable to take full advantage of information available in regional, national, and even international herbaria on the distribution in the region to be explored of those species which might be of particular interest to the collector.

It is especially important to obtain the most accurate information possible on rainfall distribution in the region to be explored, since the possibility of finding plants with mature seeds often depends on weather conditions. When traveling during rainy seasons, few mature seeds may be found. If trips are made near the end of the dry season, seeds possibly will not be found, and the plants of interest to the researcher may have been eaten by animals. If the objective is to collect as wide a range as possible of genera, species, and ecotypes of germplasm, the best time to start the trip is four to eight weeks after the beginning of the dry season.

In some cases the collector might have the opportunity to make an exploratory trip into the region of interest. Such a survey trip will provide information on the previously mentioned topics.

Finally, an important aspect of previous information refers to the breeding habit of the germplasm to be collected. To make a decision on whether to collect seed separately from individual plants or on a population basis, the collector should have an idea of whether he is dealing with self- or cross-pollinating species.

## Equipment Necessary for Collecting Germplasm

### General equipment

The complexity of the general equipment required for a germplasm collection trip depends primarily on the availability of logistical support data on the region to be explored, on the duration of the trip, and on the collector's fondness for camping. We will list only the basic elements required by the collector if he is not going to camp (if he likes camping, he will know the type of equipment he should take along):

- a. A well-equipped vehicle, possibly with four-wheel-drive, in perfect working condition; an extra gasoline tank; oil, brake fluid; essential spare parts, including equipment to fix flat tires; a basic tool kit for repairing the vehicle; a water container; other tools such as shovel, pick and machete.

b. Thermos for cold and hot drinks; water; food; medicine (including antivenom serum).

c. Personal effects including articles of personal hygiene, high boots (to protect against snake bite), hat, pocketknife.

d. Blanket, hammock, mosquito net, flashlight with spare batteries.

e. Nonessential but advisable articles such as letters of introduction and recommendation for government officials and private individuals who can cooperate; small gifts to offer in appreciation for hospitality shown.

### Technical equipment

The amount of technical equipment depends upon the number of scientists participating in a collection trip and their degree of specialization. In addition to the driver who should have good knowledge of repairing the vehicle, a team of two to three scientists, one of them a soil specialist, could be considered as ideal. Obviously, if a botanist forms part of the team, he will take with him plant presses and a drying stove for herbarium specimens (see Chapters IV, Soils; V, *Rhizobium* and VI, Plant Pests, for special equipment). If the main objective of the trip is the collection of forage plant germplasm, the following items are considered essential:

a. For the description of the collection site and identification of the material collected:

- detailed highway and road maps;
- compass and altimeter, topographic maps if available;
- marking pens with indelible ink;
- writing materials, or preferably a small tape recorder with sufficient spare tapes;
- camera and film.

b. For collecting germplasm:

**Seed** – a sufficient number of paper bags of several sizes, stapler with spare staples, staple remover.

**Vegetative material** – a small trowel and scissors, plastic bags with holes, newspaper and styrofoam box, burlap sacks, water container, white adhesive tape, plastic tags with ties.

- c. For herbarium material:
  - a large plastic bag;
  - plant presses with absorbent paper (newspaper) and cardboard;
  - magnifying glass;
  - formaldehyde.
- d. For processing collected seed:
  - a wire “cage” for storing and drying the bags containing the seeds;
  - rubber board for threshing seeds (see Figure 1, Chapter II, page 13).
  - dissection equipment (including tweezers);
  - insecticide and fungicide dust and protective masks (e.g. made of cotton).



## II

### GERMPLASM COLLECTION IN THE FIELD

*R. Schultze-Kraft*

#### **Basic Considerations**

While traveling, the collector has to make the daily decision as to where and how many times he should stop the vehicle to look for germplasm. Both the decisions as to the collection sites and the frequency of the stops depend mainly upon the collector's experience, the specific objective of the trip, and the time available.

If it is assumed that the objective of the trip is to gather the most representative samples of the genetic variability of germplasm in a very extensive region, the changes in vegetation, topography, altitude, and land use can serve as guides in determining whether a stop is advisable. In case the vegetation, topography, etc., are uniform over long distances, it might be advisable to stop and take samples every 30-50 kilometers.

Another decision the collector has to make involves collecting samples along the roadside. Since these zones often are characterized by higher soil fertility and protection of vegetation against burning and grazing, the possibilities of finding germplasm as well as its genetic variation (including products of natural hybridization) may be greater near the roads than in adjacent areas. On the other hand, because of passing traffic, the collector never can be sure whether the germplasm from a site near the road is native to that place. Thus, one of the most important notes about the collection site is to identify it as a "roadside area".

The main objective of a germplasm collection trip is the collection itself. It is better to spend the available time at a given collection site gathering a large quantity of samples than to make routine descriptions of less important characteristics of the samples collected. Unless there is a special interest in a specific characteristic, the collector should make observations only about those samples which are outstandingly positive or negative.

A small tape recorder is helpful for taking notes about the collection site and the particular characteristics of the germplasm collected. This is a time-saving

device. Others may prefer a permanently bound field notebook, although with the notebook precious daylight hours may be consumed which could be better spent in the collection of plant material.

At the end of the working day in the field the recorded notes are transcribed to the respective collection cards. A camera is another useful item for the description of the collection site and particular characteristics of the samples collected. A few well selected photographs of the site and of the samples collected will provide a valuable record.

How valuable is a given collection in terms of its apparent lack of vigor or similarities with collections previously made? The collector often has to decide whether to collect seed or vegetative material of rather poor-looking plants with an apparent lack of vigor or to sample once again germplasm which apparently is identical with a sample taken at a previous stop.

The collector should be aware that any given genotype, even though it does not look like a promising forage plant, might represent a valuable source of important genes which should be preserved. Furthermore, the appearance of a plant at a given collection site reflects only its interaction with that particular environment. On many occasions, germplasm which, at the collection site may not look promising, proves to be outstandingly vigorous in a different environment (and vice versa).

It is also advisable to repeat sampling germplasm material and to keep it separately, even though it appears to be identical morphologically to plants collected at a previous stop. There may be differences in genetic and physiological characters. Therefore, it seems better to run the risk of collecting duplicates, which eventually could be unified in the introduction plots than possibly to miss very valuable germplasm.

## Seed Collection

The following observations should be kept in mind when collecting seeds:

- a. The collector must make sure *in situ* that the samples he is taking really do contain good seeds.
- b. In case there are no completely ripe seeds, it is better to collect some immature seeds than to sample no germplasm at all. Sometimes green material already is physiologically mature and might germinate.
- c. In the case of legumes with dehiscent fruits which apparently no longer contain any seeds, the collector may be justified in looking for and collecting seeds which have fallen to the ground.

- d. The plants of stoloniferous legumes should be pulled out carefully. Some genera, in addition to the normal aerial fruits, may produce underground fruits under certain conditions.
- e. In the case of self-pollinating or apomictic germplasm, seed samples from more than one plant should be collected and may be combined as long as phenotypically they seem to belong to the same ecotype. In the case of germplasm which the collector knows or suspects to be cross-pollinating, it is preferable to collect seeds from single plants and keep them separate.
- f. The decision as to quantity of seeds to be collected from a given genotype depends on its breeding habit, availability of mature seeds at the collection site, availability of time and specific interests of the collector. Because of seed requirements for follow-up evaluation work, it may be highly desirable to collect as many seeds per sample as possible. However, the collector should not overlook the necessary and sometimes time-consuming subsequent seed cleaning and processing activities that need to be done.

It is advisable to number the collection sites consecutively and to mark each paper bag containing germplasm with the respective site number and name of the collected material. The best way to close the bags is with a stapler.

### **Collection of Vegetative Material**

Often it is necessary to collect vegetative material, especially when the germplasm in which the collector is interested has not produced mature seeds or when there are no seeds left on the plant. The time of maturation is so highly variable that it is impossible to select a season for the collection trip during which all species and ecotypes are bearing mature fruits.

With grasses, it is nearly always advisable to collect vegetative material, since it is extremely difficult to estimate seed quality and viability in the field. In the case of legumes, vegetative material should be obtained only when mature seeds cannot be found. When samples are to be taken from herbaceous or bush species that do not have a stoloniferous growth habit, the collector should look for young plants and dig them out carefully from the place where they are growing. For stoloniferous species, he should collect stolons with nodes that have strong roots.

Three alternative ways of handling the collection of vegetative material are proposed:

- a. To transplant the material dug with adequate soil into plastic bags that have holes to provide good drainage.

- b. To place the vegetative material without soil in burlap sacks that are kept wet.
- c. To place the vegetative material without soil wrapped in wet newspapers and keep in styrofoam boxes, which retain moisture for a long time.

Duration of the trip, time available, and space available in the vehicle determine which of the three methods is most practical. In any case, it is important to mark the vegetative material gathered with at least the number of the collection site. If the material is transplanted into plastic bags, it is convenient to use a white adhesive tape. As for the other two methods, good results are obtained using white plastic tags with holes so they can be tied to the collected material. In all cases, a marking pen with indelible ink that writes on plastic should be used. The collector should check from time to time to see that the vegetative material is sufficiently moist.

### **Collection of Herbarium Specimens**

Although the main objective of the trip is the collection of germplasm, unfamiliar species should be pressed so as to identify what was collected. Unless a botanist forms part of the collector's team, taking herbarium samples in the field should be limited to a minimum because of the time required. The collector may later have the opportunity to obtain herbarium specimens under greenhouse conditions or from introduction plots into which the collected samples eventually will be sown, but some species may be missed if the plants do not survive until identified.

In those few cases in which the collector definitely wants to take herbarium specimens during the collection trip, he has to decide whether it is indispensable to press the material immediately after cutting it or if it can be placed in a plastic bag and pressed when the working day in the field is over. The second procedure is more advantageous in terms of time spent at the collection site. For conservation of herbarium specimens during the collection trip, spraying them with formaldehyde after having pressed them is recommended. If collecting herbarium specimens is one of the particular objectives of a collection trip, the use of formaldehyde cannot be considered as an ideal conservation method; in this case, the use of a drying stove would be necessary.

### **Processing of Seed Collected**

In many cases, the material collected still contains too much moisture. This material consists of fruits, seeds and other parts of the plant including flowers, bracts, leaves and pieces of stems. To prevent deterioration and loss of the collected germplasm, it is necessary to dry the samples as soon as possible.

Under field conditions the paper bags with the samples may be exposed to the sun and to the air. The most adequate drying equipment is a wire cage in which the bags containing the samples are placed. Exposing the cage to the sun three to five times during the day is sufficient for adequately drying the samples. The stops made at collection sites serve this purpose.

Threshing of seed should be done as soon as possible after collecting. In some cases (*Centrosema*, *Galactia*, *Macroptilium*, etc.) this is an easy task, consisting merely of shelling the fruits. In other cases (*Stylosanthes*, *Zornia*, *Desmodium*), this is much more difficult and time-consuming, and one should have special implements to facilitate the task. Good results have been obtained using small rubber boards (Figure 1) between which the collected sample, which must be well-dried, is crushed. In this way, all material, with exception of good and mature seeds, disintegrates. Afterwards, the seeds may be separated from the crushed material with tweezers or a similar implement or, even better, by blowing on the sample.

The final but equally important step of processing the collected germplasm is to treat the seeds with an insecticide. The most appropriate method consists of

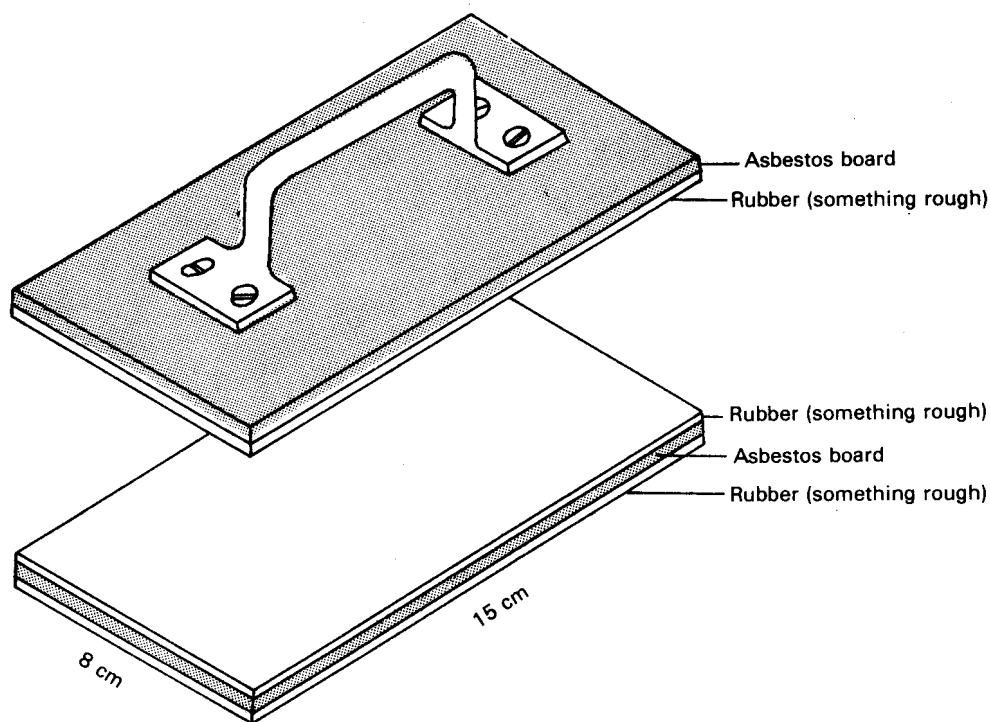


Figure 1. Rubber board for threshing and cleaning small samples.

placing the cleaned seeds in a new paper bag or envelope, where a small amount of insecticide dust is applied with a spatula on top of the seeds. Once this has been done, the bag is closed with a stapler and shaken so that all the seeds make contact with the insecticide. If the sample is to be exported to countries where sanitary regulations require that they also be treated with fungicide, it can be applied as a mixture with the insecticide.

# III

## DESCRIPTION OF THE COLLECTION SITE

*R. Reid and J.R. Lazier*

When considering the descriptors for a collection site, it is necessary to think not only of the data which appears of immediate use or relevance to the collector but also of the future needs of genetic resource workers. To meet future needs, it is important that as much site information as possible is recorded.

A plant collecting expedition usually is at a specific collection site for only a short time. Therefore, it is of prime importance that the assembling of environmental data be well-documented with ecological and agronomic information, and that these data be recorded on a standardized form for efficient and rapid retrieval. Careful selection of the descriptors will serve as a base from which changes in the sampled area may be assessed in the future.

In Chapters I and II of this manual the importance of researching the available information for collection is emphasized. The selection of collection sites should be based upon surveys and maps of climatic conditions, soil characteristics, and vegetation types. Much of this information will, of necessity, be recorded either before or after the actual collection is made, since such detailed information will not be available in the field. This makes it imperative that the collection site be located as accurately as possible. To reduce communication problems, the descriptors adopted for the entire scheme of plant collections should be clearly defined. With increasing amounts of material being collected each year, eventually only computerized systems will be able to handle the mass of information.

### **The System**

The system proposed here for the description of the collection site consists of a list of descriptors, their code numbers, the number of characters (letters or numbers) allowed for that descriptor and a dictionary of definitions of the descriptors. Appendix 1 presents a complete list of the descriptors for the

collection site, soil conditions, *Rhizobium*, insects and diseases, and for the characterization and preliminary evaluation of germplasm. In Appendix 2 those descriptors that are not self-explanatory are defined. The system, now adopted for many of the world's crop plants, is patterned after the data form for germplasm collections developed by Information Sciences/Genetic Resources Program, University of Colorado, Boulder. This system not only reduces the unwieldiness of collection site data and its retrieval, but also makes it easier for the field collector by presenting the decisions for him on a standardized format.

The system also is flexible in that any descriptor may be included or omitted on the field collector's form at the discretion of the collector or the responsible collecting institution. However, for the system to work effectively on an international basis, the same code number for any specific descriptor should be used by all organizations in the germplasm information network. Additional descriptors with their code numbers may be added as needed, but the code number should be assigned only after concurrence by all institutions collaborating in the germplasm collecting network. In this system the descriptors may be recorded in alphabetical, alpha-numeric, or numeric form.

## **The Form**

Most any type or size of form may be developed by the responsible institution and the collection crew. Some collectors may prefer a thick card for recording the descriptor information while others may prefer a specially prepared form which will provide copies of the descriptor information in duplicate, triplicate or even in quadruplicate. One copy may accompany the seed or plant sample, another may go to the computer operator, another with the *Rhizobium* sample, etc. The important requirement is that the descriptors have the same definition and code number irrespective of the language of the country in the germplasm network. Only one data form should be used for each sample representing the seeds from a single plant. Exceptions to this might be the case of self-pollinating forage species, or grasses which reproduce by apomixis, where seeds from a colony of plants might be combined for the sample. This must be left to the discretion of the collector, but a good rule to follow is, if in doubt, collect the seed from only one plant as the sample.

## **General Descriptors for the Collection Site\***

The descriptors for the collection site are listed in three categories: general information and location (descriptors 1-39); natural habitat and area vegetation (descriptors 40-59); descriptors for specific collection site (descriptors 60-69).

---

\* Appendix 1 presents a complete list of the descriptors and Appendix 2 their definitions.



# IV

## SOIL SAMPLE COLLECTION AND PROCEDURES

*L. A. León, W.E. Fenster and P.A. Sánchez*

The main objective in collecting tropical forage plants, seeds, and/or nodules is to discover and retain new germplasm which may be of agronomic importance. To retain some of the germplasm it may be important to know and understand the soil ecological system where it was collected. The purpose in taking a soil sample is to determine the chemical and physical properties of the soil in which a given plant, seed, or *Rhizobium* strain was found. When this type of information is secured for the places where germplasm is collected, it may be possible to show an interrelationship between plant occurrence and given soil properties. This information then could be used, not only for maintaining certain species in a germplasm bank but also for later propagation on a larger scale.

The purpose of this chapter is to set forth guidelines for soil sampling and processing when collections are being made for a germplasm bank.

Since it is important for the germplasm collector to obtain a representative soil sample from the plant collection site, certain materials and equipment, along with sampling, handling, and storage guidelines, are required.

### Materials and Equipment Required

The germplasm collector should have the following materials and equipment to facilitate his task of soil sampling:

- hollow-bore auger;
- small folding or garden shovel;
- geology hammer;
- knife;
- plastic washing bottle;
- metal tape measure;

- small plastic bucket;
- polyethylene bags;
- rubber bands;
- canvas bag to carry the samples;
- colorimetric equipment to measure pH.

## **Guidelines for Sampling**

### **Soil sampling**

The soil sample should be taken in the immediate vicinity of the root of the plant whose seed or other plant part has been collected for propagation. The depth of sampling should be about 15 cm. About 500 g of soil are necessary for laboratory analysis. It is important that the soil surface be scraped and cleaned for organic debris prior to sampling. In many instances the area may be quite variable and the judgement of the collector becomes extremely important in sampling the area which will best represent the soil in which the plant is growing.

### **Special conditions of the soil**

In many cases the germplasm collector must discern if the plant is growing in a particular spot because of some unusual circumstances. These could include areas near fences, roads or paths, near or in recently fertilized or limed fields, or near old stockyards, salt licks, or places where agricultural supplies are handled. It is extremely important that the soil samples portray whatever unusual circumstances led to the germplasm growing there. In such cases, the collector may want to take two samples, one from where the plant is growing and another from an adjacent area. In this way it may be possible to discern, through soil analysis, why the plant was growing in that particular circumstance.

### **Soil profile**

Whenever possible, soil profiles should be examined and the outstanding features taken into account. These could include thickness of the different horizons according to their color and texture and depth of the rooting zone. This information is in addition to that required in Appendix 2 "Descriptors of Soil Characteristics".

### **Presence of stones or rocks**

The presence, numbers, and sizes of stones or rocks and their state of decomposition also should be indicated. In this way a generalized taxonomic classification of the soils can be made, provided the different horizons are sampled. For example, this can be done when a plant has been obtained in a place near a road cut or irrigation ditch.

## **Subsoil samples**

If the plant being sampled is a shrub or bush, it will be necessary to take a separate sample of the subsoil. The depth of this sample will depend on the depth of the greatest volume of active roots.

## **Handling of the Sample**

When sampling, the soil should be placed in a clean bucket and thoroughly mixed. About 50 g of this sample then should be placed in a clean polyethylene bag. The same operation is carried out with any subsoil samples that are taken. The sample bags should be marked in exactly the same manner as those on the germplasm collected. If a sample is wet, it should be air-dried in a clean shady place.

The remainder of the sample left in the bucket can be used to carry out field observations, such as the soil texture and pH. All the observations made in the field should be entered on the "Descriptors of Soil Characteristics" form. It is most important that the sample number appearing on the form corresponds to the one on the bag containing the soil sample.

If no soil sample is to be sent to the laboratory, the soils still should be carefully sampled for making on-site field observations and determinations, all of which should be entered on the data collection form.

## **Special Precautions**

The following precautions are recommended whenever an analysis of a micronutrient is necessary (Peterson R.G. and L.D. Calvin, 1965):

- The use of galvanized, soft steel, and/or bronze tools must be avoided whenever Zn, Fe or Cu are to be analyzed. The best equipment to use in this instance would be a stainless steel auger, a plastic bucket, and polyethylene bags.
- Once the soil samples have been taken, extreme care should be exercised to avoid contamination from materials such as fertilizer dust, limestone, and ash. Paper or cloth bags are not recommended unless they have an inner plastic lining.

## **Shipment of Samples for Analysis**

The samples collected during each working day should be air-dried and packed immediately in air-tight bags. If the sample collecting is done in only one country, the samples should be sent to a local laboratory for analysis. This avoids

unnecessary delays due to quarantine regulations governing the import of plant materials and untreated soils.

In order to have comparable data, however, it is essential that all laboratories where samples are sent use the same analytical procedures, especially regarding soil pH, aluminum, essential elements, and cation exchange capacity.

### **Descriptors for Soil Conditions**

Appendix 1 presents a list of descriptors for the soil conditions and Appendix 2, their definitions.

# V

## COLLECTION OF STRAINS OF *RHIZOBIUM*

*R. A. Date and J. Halliday*

A successful legume plant at a particular site is frequently a manifestation of an especially effective symbiotic association between host plant and strain of *Rhizobium*. A plant explorer who collects seed alone should not expect to see the full potential of the plant line in subsequent evaluations unless some attempt is made to reconstitute an equally effective symbiosis. Commonly, an introduced legume fails to encounter effective native strains, and/or nodulate effectively with available inoculants, in a new environment. Experience shows that this situation can be avoided if strains of *Rhizobium* which are genetically and geographically affiliated with that host can be made available for use as inoculants. Thus nodule (*Rhizobium*) collection should be undertaken as a routine procedure by plant collectors.

This chapter is directed specifically at the plant collector who has neither microbiological training nor legume bacteriology back-stopping from within his research organization. The step-by-step instructions for collecting nodules in the field maximize the possibility of successful isolation of a *Rhizobium* strain by the laboratory which undertakes processing of the samples. A companion volume of this manual describes field and laboratory methods for isolation of *Rhizobium* from nodules, for characterizing the strains in a pure culture and for conserving strains in a *Rhizobium* collection\*. It is sufficiently comprehensive to permit a research group to initiate work in this area and would be particularly useful to a plant pathology laboratory with no prior experience with *Rhizobium* if they were to undertake to back-stop a legume collection program.

### Preparation for the Collection Trip

Timing of a trip to collect nodules for isolation of *Rhizobium* should coincide with the season of vegetative plant growth and adequate soil moisture. This may not always be possible, since combined *Rhizobium*/plant collecting expeditions

---

\* Halliday, J. and Date, R.A. Collection, Isolation, Characterization, and Conservation of *Rhizobium* Strains. CIAT, Colombia. In preparation.

usually are made at plant maturity to facilitate seed collection. Unfortunately, at this time most legumes carry only a reduced nodule complement (if any) and the dry, baked soil conditions impede excavation of roots.

It may be necessary to undertake a separate expedition specifically to collect nodules because of non-coincidence of the nodule- and seed-collecting seasons. Therefore, it is imperative that plant collection site documentation be sufficiently explicit to permit the exact location (and ideally, the same plant) to be re-visited in a different season.

## **Materials and Equipment**

The materials required during a collection trip depend on its duration:

### **Short term**

- spade;
- knife;
- permanent marker;
- field book;
- selection of polyethylene bags;

### **Medium term**

- all above;
- collection vessels (Figure 2);

### **Long term**

- all above;
- packing materials for dispatching samples to collaborators;
- sterilizing solution ( $\text{HgCl}_2$ ).

## **Collection**

When collecting from roadsides and other disturbed sites, note that soil conditions, plant performance and nodulation may not be typical of the region. Seek out legumes that appear successful in an ecosystem pertinent to your

interests, such as native pastures and/or stable natural ecosystems. Having located a plant from which to collect nodules, subsequent procedures depend very much on duration of the collecting trip.

When it is possible to deposit the sample on the same day with the laboratory which will perform the isolation, it is simplest to excavate the whole plant and its nodulated root system in soil and pack it firmly into a polyethylene bag for transport to the laboratory.

For trips of one to 14 days, collected nodules must be prevented from decomposing and protected from invasion by soil microorganisms which interfere with subsequent isolation procedures. Segments of the nodulated root (be sure it belongs to the proper plant) may be collected in a plastic bag with moist soil.

The nodules may be conserved in a vial containing a desiccant. Collection vessels (Figure 2) must have caps that provide an air-tight seal. Desiccant (anhydrous calcium chloride or silica gel) occupies one-quarter to one-third of the volume of the container. It is held in place and must be prevented from coming in contact with the nodule samples by a cotton wool plug. The volume of nodules and/or roots should not exceed the volume of desiccant.

Seldom is it necessary to excavate whole plants to retrieve nodule samples. Careful paring away of soil around the root crown or stolon nodes to locate young adventitious roots and then carefully excavating these with a penknife often yields best results. If this method proves unsuccessful, (and it depends very much on the species involved), deeper excavation of the root system may be necessary. Insert a spade vertically into the ground some 15-20 cm from the base of the plant and on all four sides so that an intact sod of soil containing the bulk of the plant root can be pried free. Extraction of the root system is made easier by immersing the sod in water and allowing the soil to fall away. Even without water, it should be possible to examine the root system for nodules by sequentially fragmenting the soil by hand.

The location of nodules on the root system is very species dependent. In a sward situation, the nodules of stoloniferous legumes usually are concentrated superficially, within 1-2 cm of the soil surface, attached to adventitious roots. In most *Stylosanthes* species they are equally distributed along the length of the primary and lateral roots in the top 10-15 cm, although in *Stylosanthes capitata* they tend to be found only at a depth of 15-25 cm.

In *Leucaena*, nodules seldom can be found under mature stands and the best hope of success is with isolated seedlings and young plants growing in full sunlight.

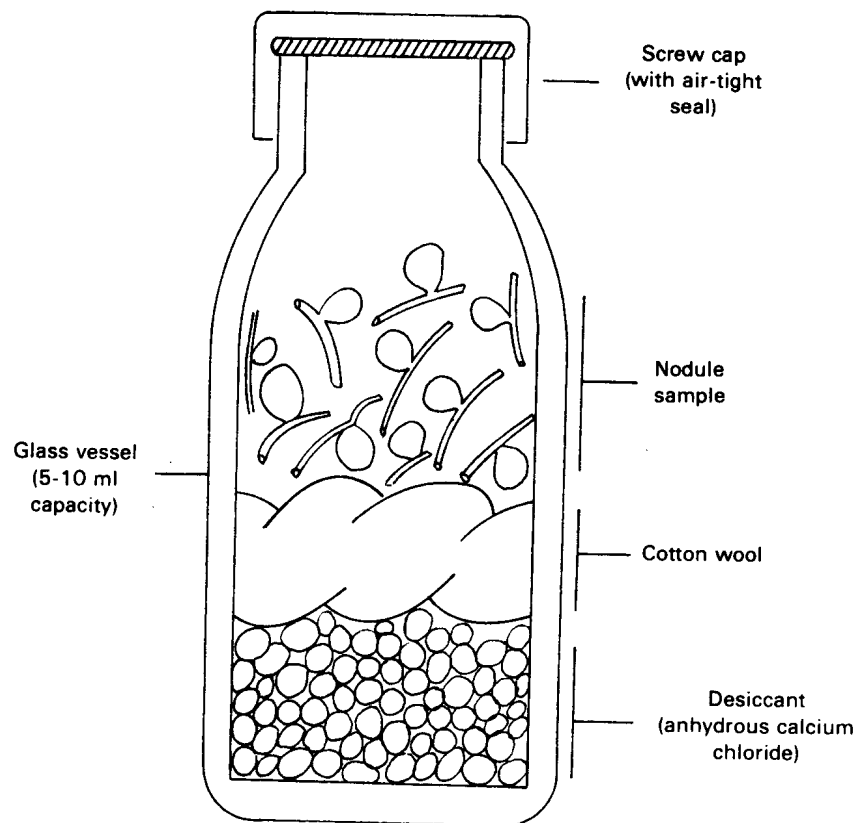


Figure 2. Vessel for storing nodules.

Do not expect to locate nodules on the main rootstock of perennial species. Nodules of most legumes have a finite life span, and a maturing root that may have borne nodules when younger, no longer has the anatomy that would permit reinfection and nodulation. Nodules on perennial species sometimes can be located by following a root out to the zone of new growth; this zone may be many centimeters from the crown. Do not expect to find nodules on a plant that has been pulled out of the ground by force. In most species, the connection between the nodule and root is extremely tenuous.

Sample only fresh, firm nodules and avoid taking samples of those which are damaged or decaying, since routine isolation procedures are unsuccessful with such material. If the root to be sampled is abundantly nodulated, section some of the nodules to check for white, pink or green pigmentation (sectioned nodules are of no value for subsequent isolation).

The inner pigmentation often is visible throughout the semi-opaque nodule periderm, making it easy to pick active nodules for sampling. Use a sharp knife or



small scissors to sever the root about 0.5 cm on either side of the site of nodule attachment. Even very fine roots can be amazingly resistant to attempts to break them with the fingers. Such efforts usually result in compression damage to the nodules.

If the root to be sampled bears only a few healthy nodules, it is more important to collect these than to section them to describe their pigmentation. Try to collect at least 10 nodules per plant. There are two reasons for this. First, it increases the chances of obtaining a viable isolate for a particular plant. Second, the proportion of nodules from a single plant that yields strains which are subsequently found effective in nitrogen fixation with the homologous host, can be quite low, as is the case with *Stylosanthes*. This is not surprising, given the range of effectiveness of strains in the soil population of *Rhizobium* that may infect a native legume species. All nodules from a single plant represent one unit of collected material and may be stored in the same desiccating tube. Under no circumstances should nodule samples from different plants be combined. Label the sample with a specimen number that relates it to the documentation of its plant accession and site of origin.

Collecting trips of more than 14 days in duration pose special problems. No definite data are available on the loss of viability by rhizobia in desiccated nodules during storage. Our specification of 14 days as a safe limit is somewhat arbitrary and probably conservative. A collector who will be away from home base for two weeks or longer may either dispatch samples to a collaborating laboratory periodically over the trip or make isolations enroute. To most collectors the former will appear the more attractive option. However, there are strong arguments in favor of isolating enroute and appropriate methodology outlined in the companion volume to this manual (Halliday, J. and Date, R.A. Collection, Isolation, Preservation, and Conservation of *Rhizobium* Strains) is, with practice, within the capabilities of plant collectors.

## Documentation

Most of the information required already will have been specified with respect to the host plant following the guidelines in Chapter III. Parameters referring specifically to nodulation are: whether or not the plant was nodulated; whether or not nodules were collected; whether or not roots were collected; and whether or not site soil was collected. As full an account as possible of the origin of each nodule sample should be forwarded to the collaborating laboratory which will perform the isolation.

## Specialized Laboratories

The CIAT Soil Microbiology laboratory is prepared to isolate, characterize and conserve strains of *Rhizobium* for actual and potential tropical forage legumes as a service to any collector, provided he authorizes the incorporation of his strains

into CIAT's *Rhizobium* strain selection program. Advantages to the user of this service are:

- a. A mechanism exists for authorizing the importation of nodule samples to Colombia without delays due to quarantine restrictions (for procedure see Appendix 3).
- b. Isolates can be made available subsequently to the collector in the form of pure cultures or high-quality peat inoculant ready for use.
- c. Data on strains in the CIAT *Rhizobium* collection for forage legumes are updated, catalogued and distributed each six months to research groups in Latin America and throughout the world. This ensures both efficient dissemination of information and maximum opportunity for access to, and utilization of, recently collected strains.

Several other laboratories are prepared to offer some of these services (Appendix 4). By using a local laboratory, a collector may reduce the risk of loss of samples in the mail. When tending to utilize these services it is essential to communicate with the collaborating laboratory at the planning stage of the collection trip. Advise them of the approximate number of samples to expect and of any special emphasis with respect to species or soil type from which material will be collected.

# VI

## COLLECTION AND PRESERVATION OF INSECTS AND PATHOGENIC ORGANISMS

*R.M. Sonoda*

Many collectors of plants for potential forage use in the tropics or subtropics probably have seen evidence of insect damage and plant diseases but usually have not had the time, technical training, or proper equipment to identify the problem or collect and preserve specimens for future identification. So far, only a few plant diseases and insects have been reported on legumes and grasses used in the tropics and subtropics, although many more probably have been seen by plant collectors. As grasses and legumes suitable for forage in tropics and subtropics are more widely and more intensively grown, disease and insect problems will undoubtedly increase. Insects and plant diseases are already important limiting factors in the growth of recently established tropical forage plants, e.g., stem borer and anthracnose on *Stylosanthes* and armyworms on grasses.

A plant collector should be aware of insects and diseases for several reasons: (1) Such awareness helps him make certain that the plant material or soil samples he collects and sends is free of, or made free of, potential pest problems (see Chapter VIII for treatment of plant material to prevent movement of pests from areas of collection to areas of introduction); (2) damage done by a pest at a collection site may serve as an early warning of potential pest problems; (3) differences in damage to different plants of the same species may be the result of differences in susceptibility to the pest.

The site of collection of plant materials serves as an important source of disease and insect problems. The introduction of an apparently minor pest along with a plant may result in a major problem of the plant under a different set of environmental conditions. The pest may be highly destructive on plants of the same species that have been selected and grown for many years in isolation from the pest and may have been selected away from tolerance to the pest. In addition, a minor pest of one plant may become a major one on plants of other species in the area to which the pest is introduced. Strict quarantine measures can keep areas isolated from potential pest problems.

---

Collaborators: Silvia Andrade, R.C. Bullock, Mario Calderón, Jillian M. Lenné.

In order to rationally prevent movement of pests, however, and at the same time promote the introduction of new genetic material, pests must be identified, and a general outline of their life cycle and their methods of dispersal must be understood.

In the following sections we present techniques that may be used by plant collectors to obtain information and specimens necessary for identifying pest problems. We are aware that most users of this handbook will be concerned primarily with collecting the plant itself. Therefore, the techniques presented will be those requiring the least amount of time, equipment, and technical knowledge but giving satisfactory results.

The plant collector is invited to use whatever techniques he feels are suited to his particular type of collection trip. However, because of the increasing importance of pests on tropical forage plants, the plant collector should be aware of pest problems at collection sites and should put as much effort as possible into collecting information necessary for identifying and evaluating the pest or problem involved.

The techniques are divided into two sections. The first section deals with photographs and note-taking in the field. Specific notes on symptoms, extent of injury, etc., will be of great importance in evaluating a problem. The second section deals with methods of collecting and storing insects and plant tissue damaged by pathogens.

## **Taking of Photographs and Collection of Field Notes**

Materials required are data cards and a good 35 mm camera with close-up lens.

### **Photographs**

It is of utmost importance that good photographs be taken of:

- a. General habitat of affected plant.
- b. Entire plant showing damage or disease symptoms.
- c. General symptoms or insects.
- d. Close-up of: (1) lesions, either external, e.g., leaf spot, stem streaking, etc.; or internal, e.g., vascular browning, pith discoloration, etc.; or (2) insect damage.

- e. Different stages of symptom development or plant damage.
- f. Different stages of pest, if present.

#### Habitat of plant affected

This information can be put on preprinted forms as described in Chapter III.

#### Description of symptoms

This information can be incorporated onto data cards used in collecting plants or on supplementary cards, cross-indexed to cards used in collecting from an area. Desirable information will be:

- a. General condition of plant; e.g., good, fair, poor, dead.
- b. Part of plant affected; e.g., leaves, stems, whole plant, flowers, fruit, and seed.
- c. Type of damage or symptoms; e.g., leaf roll, holes in leaves, leaf spot, stem-streaking, vascular discoloration.
- d. Stage of plant growth affected; e.g., young plants, old plants, fruiting plants, non-fruiting plants.
- e. Whether or not the pest occurs on some plants and not others of the same stage of growth.
- f. Estimate of injury damage (in percent) for individual plants and whole populations.

### Collection of Specimens

#### Insects

When collecting insects, care should be taken to collect the insect actually doing the damage observed. It will be better to collect several insects associated with the damage, rather than selecting one that may not be involved.

- a. **Capturing insects:** The simplest method of capturing insects is to use a net. A 30-40 cm diameter hoop generally is preferred. The nets are swung several times over or through the plants.

Another method involves placing a cloth under a plant and beating the plants lightly with a stick or spraying with an insecticide with a quick knock-down effect.

Simple traps can be made if the collector is going to be in the same area for several days. Traps can consist of substances giving off odors, a white sheet hung vertically with a source of light behind it for insects active at night, a jar or can containing bait and buried in the soil so that the mouth is even with the soil surface, and many others.

For very small insects, an aspirator consisting of a small bottle, a stopper with two holes lined with glass tubing, and two pieces of rubber or tygon tubing can be used. Air is drawn by mouth through one tube while the end of the other tube is placed over the insect, thereby transporting the insect into the bottle.

b. **Killing insects:** The kill jar is the most widely used and most convenient method of killing all types of insects. Several different poisons can be used in killing bottles. The most popular are potassium cyanide and ethyl acetate. Both compounds are poisonous to man, and bottles containing the compounds should be clearly labeled and kept in a safe place. Killing bottles can be made in several ways and be of any convenient size – 200-300 cc is standard. As a safety measure the bottom of glass jars should be wrapped with tape.

Potassium cyanide, powder or crystals, can be poured into jars and covered with 3-5 mm of plaster of Paris or circles of blotter paper. Ethyl acetate can be soaked into plaster of Paris, small pieces of blotter paper, fine cork chips, or dry boiled sawdust. (Potassium cyanide jars can remain effective for several months, but ethyl acetate jars may have to be recharged daily.)

Crumpled up newspaper can be placed in the kill jars to prevent insects from damaging each other. The newspaper should be removed when jars are recharged with ethyl acetate.

c. **Storing insects:** Members of the Diptera, Hymenoptera and some other groups usually can be stored in top quality only if they are promptly pinned in the field. Beetles and soft-bodied insects can be stored in 75% isopropyl alcohol in small-cap vials. Lepidopterous insects should be placed in paper envelopes or triangles made from a rectangular piece of paper. The paper is folded diagonally so that there is a half inch or so overlap on both edges. One edge or flap is folded over, forming a triangular envelope and the insect with wings upward is placed in the envelope and the other edge is folded over to seal the envelope. The envelopes are then placed in an insect-proof container or the paper used for the envelope may be impregnated with insecticide.

Another method of storing unpinned insects is to place them in a cigar box or similar container lined with paper. The insects are covered with layers of cellulose and another layer of insects put on top of the cellulose. Cotton wool can be placed between the top layer of cellulose and the lid to keep the insects in place. Do not let insect bodies touch the cotton wool, as body parts become entangled in it.

## Systemic disorders

In this category are diseases caused by fungi, viruses, bacteria, etc., and disorders caused by insects, nematodes, and physiological imbalance.

a. Collect as much of a plant as possible. Be sure that the plants are unthrifty or diseased but not dead. Dead plants generally are of little value in determining cause of problems, as secondary or saprophytic organisms may mask the effect or presence of the primary disorder-causing agent. If collecting a whole plant is not possible, collect representative parts of root, stem and foliage. In many cases where a plant is systemically infected, a soil sample will be informative. Collect a healthy plant or plant parts, if feasible, for comparison.

b. Be sure to check systemically infected plants to see if lesions are present in their vascular or pith region. Also note the distribution of lesions.

### Pathogens restricted to specific plant parts

a. Plant parts with lesions can be collected and kept in polyethylene bags for a day or so. If iced or refrigerated, the material will remain free of secondary organisms for a longer period. Both old and new lesions are desirable, as different stages in the life cycle of the pathogen may be present in lesions of different ages. Small samples can be placed in small bottles used for seed collection.

b. Samples of diseased parts also should be pressed if possible for longer-term storage, especially if the material cannot be examined immediately.

c. Samples of diseased area, e.g., lesions, cross-sections of diseased xylem, etc., can be killed and fixed for future sectioning. A useful killing and fixing fluid is FAA composed of 50% ethyl alcohol (95%), 5% glacial acetic acid, 10% formaldehyde (37-40%) and 35% water. Other killing and fixing agents for more specific use are available.

Cut samples into small portions suitable for microtome sectioning, with minimum bruising, compression and desiccation. For most forage plants a single-edge razor will suffice as a cutting tool. Immerse samples immediately in killing and fixing solution. Samples can be stored in small bottles of FAA until they are processed further for sectioning.

d. Fungal and bacterial pathogens can be isolated from diseased plants and kept on artificial media. Tests for pathogenicity can be made when convenient to determine the causal agent of the disease. Young lesions are preferred, as they more likely will be free of secondary or saprophytic organisms. Make isolations soon after collection, preferably in a sheltered area, e.g., tent, cabin of vehicle, etc. The isolation kit should contain: a surface sterilizing agent (a 1:10 dilution

of household bleach; NaOCl is commonly used), sterile water, sterile containers for sterilizing agent, fine scissors and forceps, a kerosene or alcohol burner, a glass rod with rounded end and test tube slopes of agar media. Single-use plastic disposable containers and test tubes usually are safer and more convenient to transport.

If fungi are suspected, cut samples with lesions into small pieces (approximately 25 cm<sup>2</sup> for leaves and pods, 1 cm lengths for stems and small roots), immerse in surface-sterilizing agent (10 minutes for 1:10 NaOCl), rinse in sterile water, and transfer pieces to agar slopes. Flame scissors and forceps before use and flame tube top before and after transfer of diseased material. Useful agar media for fungal plant pathogens include water, potato, dextrose, or oatmeal agars.

If bacteria are suspected: A small piece of tissue is placed in 1 ml of sterile distilled water in a test tube. The tissue is macerated with a glass rod and the glass rod used to streak beef peptone agar or potato dextrose agar slopes.

e. A surface imprint of a lesion area can be made if a fungus disease is suspected. An adhesive-coated transparent tape (do not use "Magic" transparent tape) is placed adhesive side down on a lesion; the tape is removed and placed on a microscope slide on which a drop of lactophenol has been placed. This is useful if spores are being produced. The slide can be examined several days later.

The most common error in collecting material from damaged plants is that not enough material is collected or only one plant or diseased part from one plant is collected. Collect material from as many plants as feasible. In all cases, material collected should be well-labeled and well-packaged. The label must be cross-referenced to notes and photographs of the disorder.

The specimens must be sent as soon as possible to a person with expertise on the problem involved. It will be much safer from the standpoint of preventing possible spread of the pest if the sample is sent to the nearest expert.



# VII

## CHARACTERIZATION AND PRELIMINARY EVALUATION

*A. E. Kretschmer, Jr.*

The objective of this chapter is to outline the procedures for the characterization and preliminary evaluation of introductions from seed germination through the development of mature plants in the field. The initial evaluation does not include harvesting for yields or the determination of quality factors, although some estimates of these characteristics may be recorded from initial plantings.

A list of descriptors for this chapter is given in Appendix 1 and their definitions in Appendix 2. These descriptors, together with their codes and definitions, will facilitate the use of a computer-assisted information management system for retrieval of information on each collection in a world-wide network. To assist in the retrieval of information, each investigator will be obliged to use the same descriptors, code numbers and definitions. However, the selection of descriptors and the format for recording the information will be at the discretion of the individual investigator. He may choose to use a field card, a notebook, or record sheet which will provide notes in duplicate or triplicate. Therefore, it is imperative that the descriptors be concise and meaningful for as wide a spectrum as possible of environmental circumstances.

The choice of evaluation procedures included in this chapter will serve only as guidelines, and modifications are expected. Procedures will differ depending on whether 50 or 5000 accessions are to be evaluated and also upon the resources available to the investigator. Preliminary evaluation of a large number of collections for desirable characteristics will provide a data base which will allow the agronomist and plant breeder to select the accessions with the greatest forage potential and reduce the number to a manageable level.

### **Germination**

Germination techniques can be critical to the preservation of germplasm when only a few seeds are available. Many times, chemical and particularly, physical barriers as well as diseases, can prevent plant establishment and subsequent seed increases.

---

Collaborators: J.B. Brolmann, J.R. Lazier, R.J. Clements, J.E. Ferguson, R.L. Burt, J.M. Keoghan, and B. Grof.

## Scarification

Because of their hard seededness, most tropical legume seeds need to be scarified to assure rapid and uniform germination. This can be accomplished chemically, mechanically or with temperature changes. Seeds should be removed from pods in some genera (*Aeschynomene*, *Stylosanthes*) prior to further treatment.

1. **Sulfuric acid.** This procedure is effective but may be dangerous to seed viability and to the person handling the acid. The object is to permit the acid to erode the seed coat but not damage the endosperm. For unhulled *Stylosanthes humilis*, seeds should be soaked for 10 minutes in concentrated H<sub>2</sub>SO<sub>4</sub>. For *Centrosema* and other legumes with more permeable seed coats, 3 minutes are sufficient.
2. **Heat.** Hard seededness can be overcome by placing seeds in water or in an oven kept at 75° to 90°C for up to 24 hours. This is very satisfactory for dehulled seeds. Temperatures should not reach 100°C. Alternating cold and warm temperatures also aid in germination.
3. **Mechanical.** The object of this method is to scratch or break the impermeable pod (if present) and the seed coat, using emery cloth or fine sandpaper to permit water and gaseous exchange. This technique is excellent for 1 to more than 100 g of seed. With fewer than 10 seeds to be scarified, seeds can be hand-rubbed on the abrasive surface, individually scratched with sharp tweezers, or affixed to masking or Scotch tape and nicked with a razor blade. For larger quantities up to about 100 g of seeds, a small electric scarifier (Forsberg or other type) or an air-blast scarifier, using 5-60 p.s.i. for two or more minutes, can be used to force seeds against the emery cloth-lined cylindrical container.
4. **Thiourea.** A 1% (by weight) solution of thiourea in water has been found to increase germination of chemically dormant *Stylosanthes humilis* seeds. The range of concentration for this species is narrow and when smaller or larger concentrations are used there is a possibility of inhibiting germination. *Centrosema virginianum* also responds favorably in most instances. If only a few seeds are available, hand scarification of individual seeds is recommended. In this case, thiourea should be used only as a last resort after observing that the swollen seeds have not germinated after about three days.

## Disease protection

It is difficult to maintain sterile petri dishes when germinating seeds, or to assure that seed-borne diseases are not present. Diseases caused by *Rhizopus stolonifer*, *Fusarium*, *Penicillium*, and *Aspergillus* can kill germinating seeds.

*Rhizopus*, because it is fast-spreading and is seed- and air-borne, can be particularly damaging. It can envelop seeds and cover the entire petri dish in a matter of days. To prevent these diseases from becoming a problem, petri dishes can be dusted with 80% wettable Difolatan, which was found to be the best chemical of eight tested for controlling a large number of diseases attacking germinating seeds. Two or three squeezes of dust per petri dish, using a DeVilbiss powder insufflator, have proven effective. This chemical, even at rates up to 1 cc per petri dish, has not been phytotoxic to *Aeschynomene americana*, *Stylosanthes guianensis*, *S. hamata*, *Calopogonium mucunoides*, and *Centrosema pubescens*. *Desmodium heterocarpon* and *D. intortum* seed germination was slowed by the high rate but not by dusting.

## Seed and Vegetative Propagation and Inoculation

There is no best method to establish seedlings for initial evaluations. Generally, when the field evaluation area is known to be weed infested and no precautions have been taken to use a preplant herbicide, it is desirable to establish seedlings in small containers before transplanting. Should there be adequate seeds, however, direct seeding to the field may be satisfactory.

a. **Petri dish.** Petri dishes lined with one or two pieces of filter or other absorbent paper should be used for germination of the smaller-seeded legumes when only one or two seeds are available. Also, they can be used successfully to germinate larger quantities of seeds. Seeds can be accounted for with this technique and the ungerminated, unswollen seeds can be air-dried and returned to the seed storage container after transplanting the necessary seedlings. With larger seed species (*Centrosema*, etc.) peat pellets (Jiffy 7 and Jiffy 9) have been used successfully. Seedlings generally should not be transplanted directly to the field from petri dishes but should be "grown-out" in other containers (listed below) prior to field transplanting.

b. **Small containers.** Square peat pots, about 6 x 6 or 10 x 10 cm, peat pellets, round pots (diameter about 5 cm), rectangular polyethylene bags, styrofoam drinking cups (7 to 8 cm diameter), and small tin cans have been used for "growing-out" seedlings transplanted from petri dishes. Some *Stylosanthes* spp. are intolerant of the high P in Jiffy 7 pellets (a P-induced Mn deficiency may occur) and with this and possibly species in other genera, Jiffy 9 pellets should be used.

Properly fertilized virgin or fumigated soil will assure healthy seedlings. A sand-based germinating medium with excellent drainage is a must for excellent germination and seedling growth. Mixtures of sand plus peat (Jiffy Mix or Jiffy Mix Plus) or peat alone also provide excellent germinating media, although for *Stylosanthes* spp., Jiffy Mix is the more preferred because of the P problem with Jiffy Mix Plus.

Sterilized soil should be used whenever possible, and if not, the soil should be carefully selected to prevent the inclusion of native legume seeds which may germinate in containers with the transplanted accessions. Care should be taken to label all pots adequately to retain the identification of plants. Numerous small size (10 to 15 cm high) permanent plastic or disposable wooden stakes are available commercially. Appropriate accession or introduction numbers can be marked on the stakes which are placed in the container with the plant.

Plastic and styrofoam propagation trays with 24 to over 100 cavities now are available. The square cavities or cells (about 4 x 4 to 5 x 5 cm) usually are about 6 to 7 cm deep. More recently, pyramid-shaped cells that maintain a normal root system are available. These are most preferred because roots continue to grow straight down without winding in a circular manner after reaching the bottom of the cell. These reusable trays are easier to use in transporting large numbers of seedlings for transplanting into the field than individual containers, although wooden trays can be made to hold the individual pots. Sections of PVC pipe 4.0 to 7.5 cm in diameter can be cut easily into appropriate lengths and placed in wooden transporting trays. Once the plants are well-established, they can be pulled carefully from the cavity without losing much soil from the roots and placed in matching sized holes punched into the soil with an appropriate probe. Plants should be grown in greenhouses, screenhouses or other protected areas and subjected to several days hardening prior to transplanting.

c. **Large containers.** Plastic, metal or clay containers having diameters and depths of about 15 to 25 cm can be used most efficiently when only one or two plants of a given accession have germinated and no seeds remain. Plants can be grown to maturity in a protected area for seed increase for later field evaluation.

Any of the above containers can be used for direct planting of seeds. This is a fast and easy method of establishment when enough scarified seed is available to plant three to five seeds per container. Once established, seedlings can be thinned to one plant per container.

d. **Vegetative propagation.** When seeds are limited, it may be necessary to propagate certain accessions vegetatively. This can be done readily with species of *Stylosanthes* and *Centrosema*. The object is to cut the growing tips or stems (or basal portions) to include at least one node buried in the soil when planted. Large containers or trays can be used for establishment of vegetatively propagated plants where seeds are not available, since 5 to 10 cuttings can be used per container. High humidity, but well-drained soil, helps to establish roots rapidly. The use of commercial growth regulators, such as indole acetic acid (IAA-0.005% by weight) or naphthalene acetic acid (NAA-0.002% by weight), may be beneficial (*Centrosema* spp.), but with most *Stylosanthes* spp. good rooting occurs within two weeks without chemical treatment.

It should be remembered that vegetatively propagated plants do not have a taproot system and their growth may not represent the same growth pattern as plants propagated from seeds. Furthermore, plants grown in any sized containers may have their normal tap and other root habits distorted because of confinement. Should plants be allowed to remain too long in their containers prior to transplanting, their root system may not develop normally when transplanted to the field. Thus, growth comparisons of field-seeded plants with field-transplanted ones may not be accurate.

e. **Inoculation.** Although in most subtropical and tropical areas of the Western Hemisphere there are an abundance of native *Rhizobium* strains that are effective on most of the Papilionideae (among exception are *Lotononis* and some *Stylosanthes hamata* ecotypes), it is recommended that seeds or transplants be inoculated. An excess of inoculant should be used to ensure inoculation. A rate of 10 to 20 cc of peat-based inoculant in 100 ml of water should be shaken vigorously for a minute and about 1 ml of the suspension added to each container soon after germination or transplanting.

If no commercial inoculant is available, nodules or roots containing active (pink color inside) nodules should be macerated in water, then diluted with more water before applying the supernatant liquid to each container. Nodules should be chosen from healthy species that are believed to be promiscuous in their *Rhizobium* requirements. Nodules from *Stylosanthes guianensis*, *S. humilis*, *S. viscosa*, *S. subsericea*, *Centrosema virginianum*, *Aeschynomene* spp., *Vigna* spp., *Macroptilium* spp., *Phaseolus* spp. are some that could be used. After several weeks, yellow or light green plants should be reinoculated by applying an excess of inoculant suspended in water.

## Field Site Preparation

No attempt will be made in this or other sections to cover sites where cultivation may not or cannot be used. The object of initial evaluation is to obtain optimum plant growth under the prevailing climatic conditions. An area with uniform soil and drainage should be thoroughly cultivated and free of live weeds, especially grasses. In weedy and previously cultivated areas Vorlex<sup>1</sup> (methyl mustard oil, CH<sub>3</sub>-N-C = S) will control soil-borne insects and diseases and kill some weed seeds. Vapam<sup>2</sup> (metham) is less expensive but does not control weeds as well as Vorlex. Methyl bromide also can be used. Other preplant selective herbicides, such as Treflan<sup>3</sup> (trifluralin) or Eptam<sup>2</sup> (EPTC), have been used with some success for weed control, while Premerge 3<sup>4</sup> (dinoseb), Dyanap<sup>5</sup>

<sup>1</sup> Nor-Am Agric. Products, Inc., 20 N. Wacker Drive, Chicago, Ill. 60606, USA.

<sup>2</sup> Stauffer Chemical Co., Agric. Chem. Div., Westport, Conn. 06880, USA.

<sup>3</sup> Elanco Products, Div. Eli Lilly, P.O. Box 1750, Indianapolis, Ind. 46206, USA.

<sup>4</sup> Dow Chemical Co., Ag. Organics Dept., P.O. Box 1706, Midland, Mich. 48640, USA.

<sup>5</sup> Uniroyal Chemical, Div. Uniroyal Inc., Elm St., Naugatuck, Conn. 06770, USA.

(naptalam + dinoseb) and Lasso<sup>6</sup> (alachlor) also have been used successfully. The use of these chemicals probably can be justified only where labor costs are high.

Another system, if hand labor is expensive or unavailable, is to use the non-specific herbicides Ortho Paraquat CI<sup>7</sup> (paraquat) or Roundup<sup>8</sup> (glyphosate). After the weed seeds have germinated, small tin cans or other containers are placed over the legume plants and the area is sprayed. An 11 liter hand sprayer can be used effectively. An SS8004 (or similar) fan-type nozzle should give good coverage. Paraquat should be sprayed only after there are numerous weeds evident, but before a majority of weeds reach the height about 2 cm. Higher weeds can be controlled effectively with Roundup. After establishment, plants need not be covered for additional spraying if care is used and there is little or no wind. Plants should not be sprayed directly. After spraying with Paraquat, the soil should not be disturbed, or a large number of weed seeds will be exposed to the surface and another germination will occur. Treatment about every two months would prevent a large weed population from becoming established. Paraquat will not kill well-established weeds or legumes. Since it is a contact rather than a translocated herbicide, small quantities drifting onto established legumes will not kill them. Roundup is translocated and cannot be applied as closely to test plants as Paraquat.

Post-planting weed control herbicides have not been thoroughly investigated, but their use could reduce labor costs and be more desirable than the use of Ortho Paraquat alone. Granular Princep<sup>9</sup> (simazine) can be applied broadcast at rates of about 1-3 kg/ha of active ingredient after transplanted legume seedlings have become established in the field. This chemical prevents weed seed germination. Sprays of Paraquat-Princep mixtures can be used successfully as a directed spray in alleyways to achieve the same purpose. Dacthal<sup>9</sup> (DCPA) can be used as a non-directed spray for *Stylosanthes* spp., *Centrosema* spp. and grain legumes, while Betasan<sup>2</sup> (bensulide) and 2,4-D also have been tried.

For mechanical or hand cultivation of weeds difficult to kill, an early start is much more desirable and efficient than late cultivation. Special attention should be given to avoid mechanical damage to legume plants. Rotary mowing and rotovators can be used to control weeds and to prevent intermingling of legumes.

Control of molecrickets (*Scapteriscus* spp.) sometimes is necessary, since they dry out soils and may damage establishing plants. A 5% toxaphene, chlordane or dylox poison bait can control these insects effectively when applied to moist surface soil at a rate of about 30 kg/ha.

<sup>6</sup> Monsanto Co., Agricultural Products, 800 N. Lindberg Blvd., St. Louis, Mo. 63166, USA.

<sup>7</sup> Chevron Chemical Co., Ortho Div., 200 Bush St., San Francisco, Calif. 94120, USA.

<sup>8</sup> CIBA-Geigy Corp., Saw Mill River Rd., Ardsley, N.Y. 10502, USA.

<sup>9</sup> Diamond Shamrock Corp., Biochemicals Div., 300 Union Commerce Bldg., Cleveland, Ohio 44114, USA.

Lesser cornstalk borers, [*Elasmopalpus lignosellus* (Zeller)] , worms and other insects should be controlled until plants have become established. Rats can be controlled using Warfarin or Pival. Rabbits may damage establishing plants and prevent proper evaluations. Wire fences have proved effective in their control, although they also may be hunted.

## Field Design

The decision to use plots of single plants, rows of plants or small plots of plants, replicated or unreplicated, depends on many factors. Although replicated plots are much more desirable for secondary evaluations, lack of seed and large numbers of legume accessions for testing make it difficult to replicate for initial evaluation and characterization purposes. It should be remembered, however, that two replications of five transplanted plants are much more desirable than a single row of 10 plants; and four replications of three plants each are better than one row of 12 plants. Although replicated designs require more labor and space, the advantages of being able to discard a large number of introductions the first or second year with confidence that the decisions are correct transcends the disadvantages.

Soil nutrient and moisture variations and missing plants that die from known or unknown causes do not result in the loss of data for one year when plots are replicated. If additional information is desired the first year, individual spaced plants within one replication can be used for different purposes, e.g., one can be harvested for yield, one used for flowering data, etc.

Regardless of plot type, it is desirable to keep legumes with similar growth habits (e.g., *Macroptilium*, *Centrosema*, *Calopogonium*, *Pueraria*; or *Stylosanthes*, *Zornia*, *Aeschynomene*) in the same area and to have one or several plots of a "standard" legume (best legume observed in previous tests) as a check in each area for comparison. With trailing legumes, for example, 'Siratro' could be planted in the experimental area. For *Stylosanthes* evaluations, 'Cook', 'Endeavour', or 'Verano' Caribbean stylo could be used; and an *S. humilis* cultivar (for annual species) could be included for comparison with newly-introduced germplasm.

Single-plant plots offer the advantage that the plant can be observed more easily and notes on rate of growth, type of root system, flowering date and other measurements are more precise. In contrast, large plant populations in rows or small plots make it more difficult to observe single plants. Alleyways between different accessions should be spaced to give the shortest possible distance between accessions without allowing the plants to intermingle. With twining type tropical legumes (*Centrosema* spp., etc.), alleyways of even 2.5 m may not be sufficient to prevent plant encroachment without chemical or mechanical control. However, in these instances a compromise usually must be made and

plants should be cut back as necessary (or the growing tips sprayed with Paraquat) during the growing season.

Yield, by cutting, can give additional agronomic information the first year and permit closer plant spacing. With small numbers of *Centrosema* or other similar trailing species, posts can be placed by each plant and the plants can be trained to grow up to the posts and tied with string when needed. This method is satisfactory for obtaining flowering information, insect or disease resistance or for breeding purposes. The method also requires less space.

Rows of convenient length (2 to 7 m or more) have been used extensively for testing introductions. These have an advantage compared to single-plant plots because the death of one or more plants does not necessarily disrupt the recording of data. Five-plant or larger plots also have been used. A shortage of seeds makes these types of plots more difficult or impossible to use. If direct field seeding is not used, increased labor and time are required. A modification of the row system, if transplanting is necessary, would be to limit each row to three to five plants spaced from 15 to 60 cm apart.

Small rectangular-shaped plots generally cannot be used for initial evaluation purposes because of low seed supplies. The advantage of these plots is that the evaluation process can be accelerated by obtaining yields and sward characteristics the first year and persistence in ensuing years.

## Data Recording

### Plant introduction

The characterization and initial evaluation program must be accompanied by good record keeping. Such items as country of origin, latitude and longitude, altitude, date of introduction, date of planting and accession number are essential to maintain continuity of the program and to prevent duplication. Requests for seeds of introductions should include requests from specific origins, especially from second-country seed supplies.

One method of maintaining a filing system for introductions is to place all the available data on permanent cards. For example, a 13 x 20 cm "Plant Descriptive Information" card is satisfactory. The front side of this card provides space for collection and evaluation site information and planting data. On the reverse side, data on growth, flowering, seeds, diseases and insects can be recorded. These cards can be placed conveniently in loose-leaf notebooks in numerical order of accession; or separate books can be used for types or species of plants, e.g., trailing, erect; or by genera e.g., *Stylosanthes* spp., *Centrosema* spp.; or in order of occurrence of the plots. Other methods of taking notes are suggested later.

If the investigator wishes to participate in a computer-assisted information management retrieval system, then he should record his preliminary evaluation



data using the coding system in Appendix 1 and Table 1. Only the descriptors which he considers appropriate for his particular situation need to be included on this form. The suggested codes for each of the descriptors should not be altered from those indicated.

In addition to the card catalog, a list in numerical order of local accession numbers can be maintained in typed form on typing paper to conform with the card catalog. A brief description, including scientific name, origin and other country's accession numbers, may be included in these lists. The lists generally aid in searching the files for the accession numbers of a particular genus or species. Also, if particular emphasis is being placed on the introduction of *Centrosema* species, an additional list can be typed to include only *Centrosema* introductions, etc.

### Field plot maps

For any type field design it is helpful to use stakes in appropriate places to mark the plants or plots. These stakes should be marked with easily read appropriate accession numbers. Wax marker pencils or paints are better than felt pens because letters are more durable. This reduces the chances of making mistakes when collecting field data, since the stake number can be checked with the number on the introduction card or plot diagram sheet when data are being recorded. There should be a plot layout map, drawn on letter-size typing paper, of the area to be planted. Included should be the accession numbers with a corresponding list of names. Enough space should be provided to record data on plant vigor, heights, flowering, etc., directly on each plot square, although later this data would be transferred to the Plant Descriptive Information cards.

This system can be used for single-plant plots or rows and small plots, replicated or non-replicated. For example, using six tropical legumes, with three replications, a plot layout as shown in Figure 3 can be drawn and numbered

REPLICATION*			REPLICATION+		
A	B	C	A	B	C
1197			418		
1926			605		
1927			1423		
1695			1778		
1697			1780		
2130			1940		

\* Upright accessions; rows contain 5 plants each, 37 cm apart and between-row distance is 2 m.

+ Twining accessions; rows contain 5 plants each, 37 cm apart and between-row distance is 3 m.

Figure 3. Example of a partial plot design for legume introductory garden.

Table 1. Example of a worksheet for recording information on establishment and flowering dates of tropical legumes (Introductory Nursery for 1976-1977, Plot 4B).

IRFL No.	Origin and Identifying Number	Date of Field Planting	Plant Status <sup>+</sup> 3/16/77	Flowering Dates					Herb. Number <sup>++</sup>
				Initial		1977			
				1976	1977	4/20	6/24	8/8	
<i>Aeschynomene</i>									
1197	Costa Rica								
	R43	7/76	S	251°	242	f'	f	f	-
1926	India PI								
	196206	7/76	SD	251°	242	f	f	f	-
1927	Rhodesia PI								
	225551	7/76	SD	251°	242	f	f	f	-
<i>Calopogonium</i>									
481	Costa Rica PI								
	227475	7/76	S	307	-	-	-	-	-
605	Australia CQ								
	562	7/76	SX	307	-	-	-	-	464
<i>Centrosema</i>									
1423	Honduras CPI								
	37273	10/76	X	#	-	-	-	-	279
1778	Bahamas *	9/76	S	265	277	-	-	-	436
1780	Bahamas *	8/76	S	243	242	f	f	f	437
<i>Desmodium</i>									
1695	India HAES								
	4530	10/76	S	#	NP	-	-	-	-
1697	Fiji Is. HAES								
	4999	10/76	S	#	NP	-	-	-	-
1799	Belize *	9/76	G	342	-	-	-	-	482

Fall flowering 1976 - began weekly observations on day 251 (from Dec. 31, 1975).

Fall flowering 1977 - began weekly observations on day 242 (from Dec. 31, 1976).

+ Plant Status:

S = survived Jan. freeze

SD = seedlings in field

X = reseeded 3/18/77

NP = no plants in field

G = in greenhouse

o Pods present at first observation

# Plants alive, but no flowering before day 363 of 1976.

' Flowering on date observed.

++ Local herbarium number of legume accession.

\* Seed collected by ARC Ft. Pierce personnel.

according to accession numbers. Because of growth habit, distances between rows for the twining legumes is greater than for the erect, ascending types.

### Worksheets

For data collection, in addition to a plot outline, a worksheet list of the introductions may be made on large-size accounting or computer-lined paper (28 x 43 cm). This worksheet would be used for typed progress reports. A list of the introductions, grouped according to species within genera in ascending numerical order, followed by the name, origin and other identifying number, should be included prior to planting. Numbered or titled columns can be keyed to indicate the different types of field data obtained (Table 1). Data obtained from the plot layout sheets can be transferred to the worksheet. The worksheet can be used the second year of observations, thus reducing paperwork.



# VIII

## TRANSFER OF FORAGE GERmplasm

*R. A. Luse*

Plant germplasm of all types has traveled repeatedly around the world, often without any control over its movement or introduction. While the early chapters of plant introduction (e.g., by the Spanish conquistadores or by the famous Captain Bligh) make for fascinating reading, it is important in the modern world that the unrestricted movement of plant materials, and the disease and/or insect pests that they may carry, be more carefully controlled.

This chapter aims to spell out the recommended steps in the efficient, but controlled, transfer of plant germplasm. These steps generally are applicable to most plant introductions but here will be specifically oriented to forages.

### **Quarantine**

Plant quarantine regulations have been set up by most countries in an effort to prevent the accidental transport of insect pests and diseases across international borders, but particularly into the given country. The rules themselves are normally reasonable and rather standardized, but the administration of regulations by government offices is sometimes inefficient and time-consuming. For these reasons, arrangements to transfer plant germplasm always should be made well in advance. It must be remembered that every sample of plant material must pass through both an export and an import step in going from one country to another. These steps will be discussed in turn, using as a general model the regulations established by the government of India, since these have been summarized very recently by Nirula (1). Compilations of national regulations have been made (2), but these are frequently out of date. The recent book by Hewitt and Chiarappa (3) should be a valuable general reference, but does not have specific chapters on forages.

#### **Import of seed material**

Here the general pattern is that material from abroad, accompanied by a phytosanitary certificate, is first inspected by the national agency responsible for

plant quarantine in the importing country. Seeds may be grown out in an isolation glasshouse and examined regularly until verified free of disease. Diseased plants and all other seeds in that lot are destroyed. Seeds from lots found to be disease-free are released to the investigators importing the seed.

The following instructions may apply, totally or in part:

1. Seeds should have been harvested from disease-free plants and be physiologically mature and dry. Small, shrunken and damaged seeds should be removed from the seed lot when received.
2. The presence or absence of organisms causing diseases (such as bacteria, fungi, and viruses) and insect pests should be determined by seed health tests. Methods used for such tests will depend on the pathogen or condition to be investigated and the seed species. Selection of method and evaluation of results requires experience in plant pathology and/or virology. General directions for such tests as recommended by the International Seed Testing Association are given in the Seed Health Testing section of this chapter.
3. Individual samples should be checked for absence of weed seeds, crop residues (e.g., leaf trash, plant debris, awns), soil particles, and foreign matter (e.g., small stones, cloth fragments). If found, such materials must be removed.
4. Seed material for routine introduction will be limited to a few hundred seeds, but for germplasm collections and breeders' population studies, the requirements may be a few thousand viable seeds. Importation of bulk seed shall be avoided as far as possible and more than 1 kg of seed should not be imported.
5. All seeds should have been inspected by the National Plant Quarantine Services of the exporting country and be accompanied by phytosanitary certificates in the form prescribed by the FAO/International Plant Protection Convention, 1951 (the so-called "Rome Certificate"), detailing treatment given and any declarations regarding absence of certain diseases as may be required.
6. All consignments, whether imported as air cargo, parcel post, accompanied or unaccompanied baggage, should be packed in such a manner as not to allow the entry into or escape of any pest from the consignment. Individual seed samples should be in sealed envelopes or cloth bags and packed carefully in such a manner that spillage or escape of pests is avoided.

7. A copy of the phytosanitary certificate should be placed in a conspicuously marked envelope on the outside of the container in which the seeds are shipped. The original phytosanitary certificate and the packing slips should be placed in an envelope inside the consignments. The packing slip should list the name of the scientist sending the seed, name of the crop, exact number of samples, number and description of packages, country of origin, and locality. Each box, carton, or bag should contain a packing slip. Usually it is stated that the seeds have "no commercial value".

It is useful for the scientist sending the seed to the investigators at the genetic resources center or other institute to send advance notice of the seed shipment. A form such as given in Appendix 5 can be used. This will permit arrangements to be made by the scientists importing the material with the National Plant Quarantine Service (to whom the seed shipments normally would be addressed).

### Export of seed material

When the germplasm collector himself or scientist having responsibility for a germplasm collection sends accessions to other countries, instructions similar to those mentioned previously apply. For example:

1. All seeds should be collected from healthy plants, free from disease. The seed should be physiologically mature and dry. Small, shrunken and damaged seeds should be removed prior to submission for clearance.
2. Seed should be taken from disease-free plants and treated against insect pests if deemed necessary. It is recommended that seed health tests as described in the respective section of this chapter be carried out as this ensures the shipment of clean seed.
3. Individual seed lots should be of uniform appearance, contain no admixture and be free from weed seeds, crop residues, soil particles and extraneous matter.
4. Seed requests should be accompanied by a form listing address of consignee, location of nearest airport and any special conditions pertaining to import. An alternative name or address to ensure clearance, such as an import permit label, is desirable.
5. Sufficient time must be allowed by the exporter for the transfer steps of (1) obtaining phytosanitary certificates, (2) shipping the seeds, and (3) inspection by the plant quarantine service of the importing country. The length of time required for these steps will vary widely among countries, but may be reduced by careful labeling of shipments and by giving advance notice to the scientist importing the seeds.

A form similar to the one reproduced in Appendix 5 may be used to accompany the seed shipment to the National Plant Quarantine Service. For purposes of reference, a list of Latin American countries (plus some Caribbean countries, Australia and the United States) and the authorities issuing phytosanitary certificates is given in Appendix 6.

It should be noted that plant quarantine regulations are sometimes unreasonable. Restrictions that prohibit seed from areas where quarantined diseases and insects are not known to exist are excessive. If no field inspection has been made and no method is known to test the seed for certain diseases, quarantine restrictions on such diseases appear to be of no value. Quarantine of disease already prevalent in the importing country is also a questionable practice. In such cases, the genetic resources center should try to eliminate such regulations by discussion with the responsible national agency. In some cases standardized regional plant quarantine regulations should be developed. Here, also, plant genetic resource centers can play an important stimulating role.

### **Registration of Germplasm**

This term generally is taken to mean the publication of the characteristics of a new plant cultivar or variety, to inform interested investigators of its features and possible advantages in plant improvement programs. Such publication can be done through certain scientific journals (e.g., Crop Science) or by means of privately circulated bulletins or newsletters. In certain countries plant breeders' rights to royalties may be applied to users of such new germplasm.

Another, more general method of recognizing the important role that national institutes have in the collection and transfer of germplasm is the use of an accession number which identifies the country of origin of germplasm. Such a number, containing the alphabetical country code (e.g. Mexico 12345, cf. Appendix 9), would be an integral part of the catalog information for a given accession wherever it is maintained in a germplasm bank or utilized in a plant breeding program.

### **Seed Health Testing**

The general methodology of testing seeds or plants for presence or absence of diseases and insect pests is outlined below. Quoted from International Rules of Seed Testing (4).

#### **Examination without incubation\***

1. **Direct Examination.** The submitted sample or a sub-sample from it is examined, with or without a stereoscopic microscope and searched for

---

\* Such tests give no indication as to the viability of the pathogen.



ergots and other sclerotia, nematode galls, smut-balls, insects, mites and evidence of disease and pests on seed or on inert matter, such as fruiting bodies, discoloration and damage.

2. **Examination of Imbibed Seeds.** The working sample is immersed in water or other liquid to make fruiting bodies, symptoms or pests more easily visible, or to encourage the liberation of spores. After imbibition, the seeds are examined either superficially or internally, preferably with a stereoscopic microscope.
3. **Examination of Organisms Removed by Washing.** The working sample is immersed in water with a wetting agent, or in alcohol, and shaken vigorously to remove fungal spores, hyphae, nematodes, etc., intermingled with or adhering to the seeds. The excess liquid then is removed by filtration, centrifugation or evaporation and the extracted material examined by compound microscope.

#### Examination after incubation

After a specific period of incubation the working sample is examined for the presence of, or symptoms of, disease organisms, pests and physiological disturbances on or in the seeds and on seedlings. The examination may be superficial or internal. Three types of media are commonly used.

1. Blotters are used when it is required to grow the pathogens from the seeds or examine the seedlings. The seeds, with or without pretreatment, are spaced during incubation so as to avoid secondary spread of organisms. Light conditions calculated to stimulate sporulation of fungi are supplied when appropriate. Germination inhibition by chemical or other means is sometimes desirable. Some pathogens can be identified without magnification, but a stereoscopic or compound microscope often is necessary for identifying spores.
2. Sand, artificial composts and similar media can be used for certain pathogens. The seeds, usually without pretreatment, are sown suitably spaced in the medium so as to avoid secondary spread of organisms and incubated in conditions favorable for symptom expression.
3. Agar plates are used to obtain identifiable growth of organisms from seeds. Careful sterility precautions are required. The seeds, normally after pretreatment, are spaced on the surface of sterilized agar and incubated. Characteristic colonies on the agar can be identified, either macroscopically or microscopically. Lighting often is useful and germination inhibitors may be used.

## Examination of growing plants

Growing plants from seed and examining them for disease symptoms is sometimes the most practical procedure for determining whether bacteria, fungi or viruses are present in the sample. Seeds from the sample under test may be sown, or inoculum obtained from the sample may be used for infestation tests with healthy seedlings or parts of plants. The plants must be protected from accidental infections from elsewhere and conditions may require careful control.

## Other techniques

Specialized methods involving serological reactions, phageplaque formation, etc., have been developed for some disease organisms and may be used.

## References

1. Instructions for Import and Export of Seed Material, Compiled by K.K. Nirula, ICRISAT, Hyderabad, India, 1977.
2. USDA Export Certification Manual, Vols. I and II, U.S. Department of Agriculture, Plant Quarantine Division, Washington, D.C. (Contains rules from 1950's to date, as revised).
3. Plant Health and Quarantine in International Transfer of Genetic Resources by W.B. Hewitt and L. Chiarappa, Chemical Rubber Company, Cleveland, Ohio 44128, U.S.A.
4. ISTA Handbook on Seed Health Testing, available in the form of Working Sheets from the International Seed Testing Assoc., Box 68, 1432 As-NLH, Norway. See also Seed Sci. Technol. 4, no. 1 (1976), pp. 31-34, 152-155, for the most recent International Rules of Seed Testing.

# IX

## PRESERVATION OF FORAGE GERmplasm

*R. A. Luse*

The need to preserve the world's present supply of plant germplasm has been emphasized repeatedly in recent years (see, for example, O. H. Frankel and J. G. Hawkes). Much of the resultant effort to preserve these genetic resources has been directed to those cereal crops upon which man has come to rely—quite literally—for his daily bread: wheat, rice and maize. Considerably less effort has been directed to the preservation of the forage germplasm upon which man relies to feed his animals and hence, indirectly, himself.

Yet the loss of native pastures, both forage legumes and grasses, has occurred wherever man has expanded his activities of crop cultivation or, increasingly, urban construction. In areas where fragile pastureland ecologies are subject to drought, over-grazing and the encroaching deserts have destroyed native pastures and the resultant forage germplasm. These are not easily replaceable, since adaptation to difficult conditions can require centuries.

While the loss of the world's forage germplasm has not reached a critical stage, its increasing rate of erosion comes at a time when new emphasis is being placed on improved pastures as a means to higher livestock production. Hence the need to assemble and collect—and then to preserve—the genetic diversity still present in the forage legumes and grasses. It is this diversity that will permit the most efficient and rapid improvement of pasturelands in vast areas of the world.

The purpose of this chapter is to present in some detail the techniques recommended for the preservation of forage germplasm, both by repeated propagation of vegetative clones and by storage of true seed. Considerably less is known about the preservation of forage germplasm than, for example, rice, and much more research is needed to raise the level of knowledge about forages to that of cereals.

## Vegetative Propagation

For those forage species not producing true seed, or at least not producing them in quantity, propagation as vegetative material is necessary. Such propagation should be carried out under the same high standards of plant sanitation as would be observed in seed multiplication, i.e., control of weeds, insects and diseases (including viruses). Plants showing disease and virus symptoms should be rogued from field plots or removed from the greenhouse. It is recognized that such vegetative propagation is laborious and may require numerous experimental plots, but the maintenance of the germplasm through vegetative clones is well worth the effort.

Field plots of a size 1 x 3 m are adequate for maintaining most forages, though larger plots would have to be established when heavy demands for the accessions are made or anticipated. In the early stages of multiplying a new accession when there is very little plant material, growth in a glasshouse or meshhouse is recommended to avoid accidental loss of valuable materials. Planting in pots or flats is appropriate and initial propagation under high humidity, as obtained from a mist chamber, is recommended. Use of hormones to obtain good initial root development is also of value. For more detail, see Chapter VII.

It is hoped that in the future the technique of meristem tissue culture can be applied to forage germplasm, so that vegetative clones can be subdivided extensively, grown as small plantlets in test tubes or flasks, and then maintained as homogenous populations free from disease and insect attack. Subjecting the plantlets to conditions of limited nutrients (probably sugars) may allow for slow growth, so that the need for handling the plant material is minimized. Adaptation of the tissue culture technique for forages should be of great value to genetic resource centers.

## Storage of Seed

### Pre-storage considerations

The quality of the seed being preserved in a germplasm bank is of great importance. Poor quality seed is likely to lose viability more quickly than good quality seed, even in good storage conditions. Here the factors most important in seed quality are:

- a. **Mechanical damage** caused by physical force during machine harvesting of the seed or by blows during hand harvesting. Such damage breaks the seed coat, causing cracks that lead to attack by fungi and bacteria. Such damage should be strictly avoided by careful harvesting by hand or with special roller-type threshers.

- b. **Physiological immaturity** usually results from early harvesting that is demanded by wet weather or desired in order to avoid seed loss after shattering of mature pods. Unfortunately, the seed spikelets in many tropical grasses are predominantly immature, even when the stalk is ripe and ready for harvest. In seed multiplication plots for germplasm material, harvesting should be delayed as much as is practical, since there is evidence that mature seed remains viable longer than immature seed. Additionally the seed should be kept in the panicle after cutting but before threshing, in order to mature.
- c. **Improper seed drying** frequently results from the desire to dry quickly the moist, immature seed before it spoils in storage. Rapid drying at relatively high temperatures (e.g., over 75°C) causes drastic losses in seed viability. However, drying at 40°C is reasonably safe for most crops and can bring seed moisture content to 11-12 percent. It has been found in *Panicum maximum* that gradual drying over a period of three to four days retains viability at an acceptable level. Reducing the drying period to one day reduces seed viability by three-fold (J.M. Hopkinson, R.L. Harty, B.H. English, 1977, pers. comm.). It is possible, however, to raise the temperature of the drying air (e.g., to 60°C) as the seed moisture content declines without affecting seed viability.
- d. **Other factors**, such as seed size and protein content, may affect subsequent viability and vigor, but these are not easily manipulated experimentally. The former factor is somewhat related to seed maturity, while the latter seems related to the nutrient status of the plant. Hence, harvesting of mature seed from well-fertilized seed multiplication plots helps insure better seed quality. Following harvest, it is assumed that the dried seed lots will be cleaned of weed seed, dirt, insect-damage seeds, split and broken seed and other contaminants, so that only **pure seed** remains in the samples taken for long-term storage.

#### Optimal storage conditions

It has been well-documented over the last three decades that maintenance of seed viability can be extended—often dramatically—by storage at low temperature, low seed moisture content and reduced oxygen concentration. The occasional case where a few seeds have by chance been stored in cold and/or dry conditions for long periods of time (even centuries) and yet still retain their viability attests to the fact that seeds are a remarkably stable storage unit by which to keep genetic information. Less dramatically, more quantitative studies have shown the value of low temperature, low humidity and low oxygen for preserving seeds with low loss of viability. Consideration of these factors led Roberts (2) to propose the following equation to relate the mean period of seed viability to the temperature and moisture content at which the seeds were stored:

$$\log \bar{p} = K_v - C_1 m - C_2 t \quad \text{where}$$

- $\bar{p}$  is the mean viability period, under most circumstances the time taken for 50 percent of the seed to lose viability;
- $m$  is the seed moisture content determined by the methods recommended by the International Seed Testing Association;
- $t$  is the temperature in degrees Centigrade; and  $K_v$ ,  $C_1$  and  $C_2$  are experimentally determined constants, with the values 6.531, 0.159 and 0.069, respectively, for rice.

This equation has been shown of value in estimating the viability period of a number of species over temperature ranges from 0° to 40°C and moisture contents from 5 to 25% (fresh weight basis). Nomographs based on the equation have been devised for rice, wheat, barley, broad beans, and peas to estimate either (1) the time period in which seed viability will fall to a certain level at any given temperature and moisture content, or (2) the various combinations of storage temperature and moisture content that are necessary to maintain seed viability above a certain value for a given period.

Unfortunately, work has not yet been done with even the major forage species to a point where the experimental constants of the Roberts equation can be determined and easy-to-use nomographs designed. Boyce and Crawford (1976, pers. comm.) report the initiation of long-term experiments in which the following genera will be tested for viability after storage at 4°C and -10°C: *Astragalus* (spp.), *Lotus* (5 spp.), *Medicago* (44 spp.), *Trifolium* (46 spp.), *Trigonella* (12 spp.). Similar experiments are needed for the tropical legumes currently of interest in national and international programs.

However, it is possible to recommend at this time what are considered to be excellent long-term seed storage conditions. The International Board for Plant Genetic Resources (IBPGR) has summarized these as follows (see Appendix 7 for more detail):

- a. Temperature maintained at -20°C, though -10°C may be used.
- b. Seed moisture content at about 5% (fresh weight basis).
- c. Seeds kept in sealed containers (glass jars, metal cans or laminated foil packets).

Not all institutes or national germplasm centers have access to -20°C cold rooms at the outset of their operations. Setting up short-term storerooms maintained at 0°C or even 10°C through the use of air conditioners or portable refrigeration units is a practical first step that may preserve valuable materials

for a number of years. In similar manner, it may not be practical (or even desirable) to dry forage seed down to 5% moisture content, and 8% may prove perfectly acceptable. There is, however, no excuse not to use sealed containers, as these eliminate the accidental regaining of moisture when refrigeration fails. The use of laminated foil packs is highly recommended, as these are inexpensive, unbreakable and easy to store\*.

Drying of seeds can be accomplished simply and without need for expensive dehumidifying equipment by means of drying cabinets and silica gel desiccant. At CIAT, a system has been developed in which 2000 g of large sized seeds (*Phaseolus vulgaris*) can be reduced from an initial moisture content of 15-18% to less than 7% in seven days' desiccation at 26-28°C over silica gel. Smaller forage and grass seeds should dry even more quickly. A laboratory desiccator of glass and stainless steel with gasketed door and stainless steel racks was used (cost US\$150), but a less expensive locally constructed cabinet should be suitable.

It should be noted that the methods for determining seed moisture content have been defined precisely by the International Seed Testing Association (ISTA) and should be followed in all genetic resource centers. Briefly described, the method for forages is as follows (cf. International Rules for Seed Testing 1976).

- a. Grind a 4-5 g sample in a mill that will produce a fine powder without heating the sample.
- b. Dry duplicate sub-samples of the ground material in an oven at  $130 \pm 3^{\circ}\text{C}$  for 1 hour. Use tared weighing containers (glass or aluminum) with tight-fitting covers; weigh containers and sample to the nearest mg (0.001 g).
- c. After drying, place cover on container and let cool.
- d. Weigh dried samples and calculate moisture content by the formula:

$$\text{Moisture content} = (M_2 - M_3) \times \frac{100}{(M_2 - M_1)} \quad \text{where}$$

$M_1$  =weight in grams of the container and its cover ;

$M_2$  =weight of container, its cover and the ground sample before drying;

---

\* Laminated packs of size 12 x 6 cm up to 25 x 50 cm (and larger) can be obtained with 5 lines of printed information (e.g. accession no. \_\_\_\_\_, species name, storage date \_\_\_\_\_, initial weight \_\_\_\_\_, seed removed: Date and amount \_\_\_\_\_) from the Disbrow Envelope Corp., 25 Linden Avenue East, Jersey City, N.J. 07305, U.S.A. Also laminated aluminum foil bags of different sizes are available from Kalle, P.O. Box 9165, D-6202 Weisbaden-Biebrich, West Germany.

$M_3$  =weight of container, its cover and the sample after drying.

- e. The difference in moisture contents determined on the two sub-samples should not exceed 0.2% (absolute value). Report the moisture content as the arithmetic means of the duplicate determinations.

## Maintenance Testing

At both the beginning and at appropriate intervals during storage, the individual seed lots (germplasm accessions) must be tested for their viability. Basic to the preservation of plant germplasm is the principle that as seed viability decreases with storage time the frequency of mutations in the remaining viable seed increases to a point where it is no longer truly representative of the original material. To minimize these gradual changes in genetic composition of the seed population, the working rule is to re-multiply the seeds when the viability of the lot falls below 90% or for those "difficult" species with lower initial viability, when the viability drops by an easily detected 10%.

Viability is determined in either of two ways, the methods for which are precisely spelled out in the ISTA International Rules for Seed Testing. These may be summarized as follows:

### Germination tests

These may be carried out using various media (paper, sand, soil) and in any type of germination cabinet that provides high humidity and control of temperature at the necessary level. Expensive equipment is not required, but care is essential in running the test and in the examination of seedlings.

Seeds may be planted in clean washed sand or in a fertile, homogeneous and sterilized soil, but for convenience in measuring seedling root development and in maintaining uniformly high humidity, the use of blotting papers is recommended. Germination may be carried out between two sheets of germination paper (later made into a two-ply roll after laying out the seeds), or on top of blotting paper placed in trays or on wax paper to retain moisture. In either case, 25 or 50 seeds should be used per 25 x 38 cm (10 x 15") paper. Here a counting board (with or without vacuum pickup) is a convenient accessory to space uniformly the required number of seeds. Use of de-ionized water for moistening the papers is recommended. After the seeds have been arranged on the paper, germination is carried out in a cabinet which provides constant or alternating temperatures and high relative humidity. Light from cool white fluorescent bulbs is recommended for the germination of some seeds.

Appendix 8 summarizes the ISTA recommendations for germinating certain forage legume and grass seeds, although the list is by no means complete for tropical forages. Chapter VII offers some additional methods for improving the



germination of forage seeds (e.g., scarification). Where seed coat permeability permits, or following scarification, the processes of moistening the seed and then chilling at 4°C produce a "stratification" within the seed that may break dormancy and increase germination. Chemicals other than nitrate (e.g., nitrite, gibberellic acid, sulfhydryl reagents) have been found to break dormancy in some species and should be tested further for forages.

After the appropriate germination period, seedlings are examined for presence of abnormalities which may be of several types, e.g.:

- Primary root thin and weak, short or long (Ic)\*
- Primary root split longitudinally, or damaged with adventitious and lateral roots weak (Ilg)
- Hypocotyl short and thick, or twisted, or curled over, or watery (IIa)
- No primary leaves, with or without shoot apex or axillary buds, or with more than half the total area of the primary leaves missing or not capable of functioning normally, or with one primary leaf and evidence of damage to the shoot apex (IIg)
- Coleoptile and primary leaves spindly, or pale, or watery (IIIe)
- Grey cotyledons (IVf) or swollen and blackened (IVg)
- Decay in cotyledons, hypocotyl, epicotyl or stem, primary root (Va, b, c,e)

In addition to abnormal seedlings, all seeds truly dead (not just in dormancy) are counted. Germination is then taken as the number of normal seedlings (N) divided by the total number of seeds tested in that lot (T), expressed as a percentage, or

$$\text{germination} = 100 N/T = 100 (T-A-D)/T \quad \text{where}$$

A = number of abnormal seedlings and

D = dead seeds.

### Biochemical tests

These are carried out in order to determine quickly the viability of seeds which normally germinate slowly or which show dormancy. The commonly used "tetrazolium test" relies on the color formation produced when the colorless

---

\* Numbers and letters in brackets refer to items in the full ISTA list of categories, which should be consulted.

compound 2, 3, 5 - triphenyl-tetrazolium chloride is hydrogenated ("reduced") by processes that take place in living cells to form a red, stable, non-diffusible substance, triphenyl-formazan. Thus, it is possible to distinguish the red-colored living parts of seeds from the colorless dead parts. Seeds may range from completely stained viable seeds to completely unstained non-viable seeds, through a variety of partially stained seeds. The position and relative size of the unstained areas in the embryo and/or endosperm – not the intensity of the red color – determine whether seeds are classified as viable or not. It must be noted that the tetrazolium test is not valid for previously germinated seeds, even if these have been redried.

The tetrazolium test involves soaking the seeds in a 1% solution of 2, 3, 5 - triphenyl-tetrazolium chloride (or bromide) at 30°C in complete darkness for a period found appropriate for the species being tested. The seeds are then rinsed with water and examined while wet. Each seed is inspected and scored as viable or non-viable on the basis of its staining pattern. The ISTA Rules for Seed Testing 1976 do not give specific directions for the tropical forages, but the instructions 6 5.2.A.26 (for *Medicago* spp., *Trifolium* spp., etc.) may be used as a guide:

1. Soak seeds in water for 18-20 hours, taking note that the seeds of certain species must be removed from the pericarp before soaking.
2. Take out swollen seeds and immerse them in tetrazolium solution for 24 hours.
3. Record the number of seeds that remained hard, and cut off a small piece of the seed coat at the end opposite the radicle. Soak these seeds in water until swollen and immerse them in tetrazolium as in step 2.
4. Decant the tetrazolium solution from treated seeds and rinse the seeds with water. Spread the seeds out for examination but keep them moist.
5. Slit open the seed coat from the embryo, from the attachment of the cotyledons to the hypocotyl-radicle axis in the direction of the radicle tip. Note the number of broken seeds, i.e., those with embryos showing no connection between the radicle and both dicotyledons.
6. Examine each seed, scoring as viable those seeds where there is one of the following:
  - Completely stained embryo.
  - Embryo showing an unstained spot at the radicle tip extending to not more than 1/2 of the radicle length.

- Embryo showing unstained spots covering half the two cotyledons opposite their attachment to the hypocotyl-radicle axis at the utmost or at the cotyledons edges.
  - Embryo showing unstained spots at the inner side of the cotyledons only in the lower parts (in the parts opposite their attachment).
  - Cases b, c and d combined.
7. To calculate viability add together the seeds evaluated as viable from both the swollen and the hard seeds. Report in addition the percentage of hard seeds and how many of them are viable.

## **Control of Inventory and Test Data**

It is evident that in any active germplasm bank very numerous data on the status of the seed stocks are generated, which data must be readily available for reference. The results of seed germination and/or viability tests done periodically during seed storage also become numerous over a period of time and are likely to be lost unless a more or less automatic system is established.

For these reasons, it is strongly recommended that in the early stages of planning for a genetic resource center or germplasm bank, consideration be given to a system of computer-assisted record keeping for all seed inventory information. Such data should include the quantity of seed currently held in each accession, the date of its last test, and the seed germinability or viability at that test. Such data files are discussed in Chapter X. It also is convenient that a system be developed that will warn when seed stocks are running low or when seed viability has dropped to a level where multiplication of that seed lot is required. The EXIR/TAXIR system has been developed with the support of the IBPGR and can meet all of the above requirements.

Advice on a system for a specific germplasm collection can be provided by the Information Services/Genetic Resources Program, University of Colorado, Boulder, Colorado 80309, U.S.A.

## **International Cooperation**

Forage germplasm collections, even those in private universities and national institutes of agriculture, should be considered a resource of value for all mankind. As such, efforts should be made to publicize the nature of the accessions, so that interested, qualified investigators or regional genetic resource centers can request small samples of germplasm considered useful for plant improvement or introduction programs. To facilitate the transfer of information (and subsequently, the transfer of germplasm), it is recommended

that catalogs, or at least descriptions, of the collection be prepared and distributed upon request. Copies should be sent to the IBPGR Secretariat, FAO, Rome, where they can be publicized via IBPGR Newsletter and other means.

It is useful, to prevent a chance disaster from destroying a valuable germplasm collection, that arrangements be made to store duplicate samples of a collection in a second genetic resource center. Such duplicate collections are not distributed generally, as this is a responsibility of the originating center, but they are available when needed by the first center or, if by prior agreement, the second center has taken regional responsibility for a world collection. The second center normally is not responsible for seed multiplication of the accessions in its duplicate collection. Planning has begun for the housing at CIAT of a duplicate set of the collection of tropical forage legumes now held at CSIRO, Townsville, Australia, so that this germplasm may be more readily available in Latin America.

## REFERENCES

1. Crop Genetic Resources for Today and Tomorrow, O.H. Frankel and J.G. Hawkes, Ed., Cambridge University Press, 1975.
2. Viability of Seeds, E.H. Roberts, Ed., Syracuse University Press, 1972.
3. International Rules for Seed Testing, ISTA, published in Seed Sci. Tech. 4 (no. 1) 1976. Available in English, Spanish, French and German versions from ISTA, Box 68, 1432 As - NLH, Norway.

# X

## DATA MANAGEMENT OF FORAGE GERmplasm

*L. Song and K. Rawal*

Two important points made in the introduction of this manual, in relation to data storage – preservation and retrieval – need to be re-emphasized. First, it is imperative that a form of data management for plant genetic resources be adopted right from the beginning, in anticipation of increasing numbers of accessions over time. Second, some standardization of data recording systems would be desirable and this should be attempted in forage germplasm banks, since these are just commencing to escalate.

In the various parts of genetic resources centers activities (such as collection, storage or maintenance, evaluation and distribution) information collected should be recorded in a form which would lend itself to easy retrieval and interpretation.

Such information, properly managed, would then act as a bridge between the germplasm itself and the users at large. This would result in the availability of information to the germplasm collector as well as others who also work in the same field.

### **Data Recording and Coding**

The first step in data management is the actual collection of the information. This aspect has been covered in most sections of this manual, using a form that was developed by the Information Services/Genetic Resources Program (or IS/GR Program), University of Colorado, Boulder, Colorado. For instance, Chapter III described how this form could be used in data recording during field collection. One advantage of this form is the ease of double checking the information coded. Another advantage is the free choice of the amount of information one wishes to record in the field, bearing in mind that the primary

---

Collaborators: J. Engels, G. Mendoza, C. Shock, A. Kretschmer Jr.

aim of going to the field usually is to collect materials rather than all the information. Once this information has been collected, it can be coded directly onto the respective right hand side of the page. This information then could enter the computer either on a fixed or free format system. The use of a free format system offers advantages in this case, since the amount of information collected in the field is variable. Thus, by entering a value (information collected) against an identifier (descriptor number), each sample will carry with it its own set of collection data. This would provide the flexibility needed for recording any amount of information about the sample at a given time.

Data entry into the computer presents the next step that needs to be improved. Very often coded data are key-punched onto 80 column computer cards. This presents little problem if the information can be systematically checked for errors. However, in recent years portable electronic recorders capable of alpha-numeric recording are being developed that should permit direct entry and storage of information on site. This equipment presently is being tested for its application for handling germplasm data in some centers (e.g., at Washington State University). Direct data entry also is possible and inexpensive through the use of various computer terminals, and several types of mini-computers may serve as interim data entry devices.

## **Standardization of Data**

In developing the "language of communication" for data among genetic resources scientists, great emphasis should be placed on the definition of descriptors actively used by the scientists. Such an objective eventually would lead to standardization of data which then could be easily interpreted by users at large.

Uniformly consistent and reliable data can be obtained if the scientists involved with data gathering follow standard definitions and rules of measurement. For instance, the use of a standard reference system (e.g., illustrations, codes, scales, etc.) could solve some of the problems created by languages. A very good example of this is the use of a 6-letter code to denote country of origin of germplasm (Appendix 9).

However, the situation with tropical forage germplasm would be much simpler if all relevant descriptors and their definitions could be delivered to all users as a "package". As a first step in this direction, it is proposed to utilize the list of descriptors and their code numbers summarized in Appendix 1 (Descriptors of Forage Germplasm Resources) together with their definitions in Appendix 2 (Definitions of Descriptors of Forage Germplasm Resources). Those who are interested in sharing their data should follow this list of descriptors, their respective code numbers and their definitions. In this respect this manual then would serve as a guide in the collection and preparation of tropical forage germplasm data. When, in some cases, certain descriptors and/or their

definitions are not defined in the same way, additional notes should be made available. It is hoped that all participants of this information network would follow a common system as outlined in this manual.

## **Information Updating and Retrieval**

The information system also must allow the genetic resources data to be added, deleted or updated and should lend itself to data retrieval and analysis. Thus, users should be able to interact with the system through a natural language (e.g., English, Spanish, Portuguese), and not have to be concerned with the computing languages (e.g., FORTRAN IV, BASIC, COBOL) in locating germplasm materials throughout the network.

Considerable progress has been made in this direction of uniform information retrieval. Computer programs now are available and being used in a number of germplasm centers that can perform information retrieval, in particular the Executive Information Retrieval Program (EXIR) which was developed at the University of Colorado at Boulder. This program also is available in smaller packages for certain mini-computers. There also are a number of other retrieval packages in existence: at the John Innes Institute a system has been developed for the *Pisum* collection, while at the Institut für Pflanzbau Staatgutforschung der FAL in the Republic of Germany, the GOLEM system has been used. The packages EASYTHRIEVE (used by USDA) and INFOL (used by CSIRO, Australia) are other programs that could be mentioned. In addition to these, there also are quite a number of computer packages (e.g., SAS, SPSS) that could do statistical analysis of data. Although the number of computer packages is many, there is a growing need to have smaller, inexpensive and widely applicable computer programs specially built for germplasm work.

## **Access to Information and Communication**

Access to all the data collected by the participants of the tropical forage germplasm network can be facilitated by the creation of data services center(s) equipped with a computer-assisted information system. Such a center most likely, but not necessarily, would be associated with the actual sites where collections of materials are maintained. The center would assist data management and organization by providing facilities, staff, and the necessary expertise in information systems. Operations such as data recording, data analysis, data retrieval, and data reporting would receive major attention. The center would allow germplasm scientists to become less encumbered by those time-consuming tasks associated with manual data processing. The center also could teach the scientists how to simplify their own data handling procedures.

Communication of germplasm information is basic to the concept of such a center. Communication facilitated by such a center will allow easy and efficient access of materials. In addition to processing specific requests for data analysis,

such a center also would provide several reference documents of more general interest to the genetic resources community. For instance, announcements could be provided for updating information of germplasm available, as well as on new accessions of special interest to breeders and other scientists. Other communication documents could involve publication of general data summaries of tropical forage germplasm held at various places. Such communication documents also should include crop specific directories which are extremely detailed documents based on the common descriptors lists, their definitions, and data collected.

Methods which provide easier access to information and communication help make people aware of the existence of tropical forage germplasm and the state of its collection in various places. Information on such germplasm, if carefully recorded, brought up-to-date from time to time, and easily retrieved for distribution, certainly would add a new dimension to the value of the germplasm collected.











316	Latitude: Dg, Min N or S	316		:		Dg		:		Min			N or S
317	Longitude: Dg, Min E or W	317		:		Dg		:		Min			E or W
318	Altitude, m	318		:	:	:	:	:	:	:	:	:	:
319	Soil texture	319		:	:	:	:	:	:	:	:	:	:
320	Soil surface color	320		:	:	:	:	:	:	:	:	:	:
321	Soil drainage (1-7)	321		:	:	:	:	:	:	:	:	:	:
322	Soil pH soln (1-4), pH	322		:	:	:	:	:	:	:	:	:	:
323	Soil salinity, mmhos/cm	323		:	:	:	:	:	:	:	:	:	:
324	Soil P (ppm), specify extract	324		:	:	:	:	:	:	:	:	:	:
325	Soil Ca (meq/100g), specify extract	325		:	:	:	:	:	:	:	:	:	:
326	Soil Mg (meq/100g), specify extract	326		:	:	:	:	:	:	:	:	:	:
327	Soil K (meq/100g), specify extract	327		:	:	:	:	:	:	:	:	:	:
328	Soil Al (meq/100g), specify extract	328		:	:	:	:	:	:	:	:	:	:
329	Effective CEC (meq/100g), specify extract	329		:	:	:	:	:	:	:	:	:	:
330	Al saturation %	330		:	:	:	:	:	:	:	:	:	:
331	Other nutrients: Nutrient, amount	331		:	:	:	:	:	:	:	:	:	:
332	Fertilizer applied: kg element/ha	332		:	:	:	:	:	:	N	:	:	P   : :   K
333	Lime applied (Calcic or Dolomitic kg/ha)	333		:	:	:	:	:	:	C or D	:	:	:
334-339	Open												

### DESCRIPTION OF PLANTING PROCEDURES

340	Planted in laboratory, day - month - year	340		:	:	:	:	:	:	:	:	:	:
341	Planted in glasshouse, day - month - year	341		:	:	:	:	:	:	:	:	:	:
342	Planted in field, day - month - year	342		:	:	:	:	:	:	:	:	:	:
343	Plot configuration, size	343		:	:	:	:	:	:	:	:	:	:
344	No. of replications	344		:	:	:	:	:	:	:	:	:	:
345	Seedling survival	345		:	:	:	:	:	:	:	:	:	:



391	Shattering resistance	391		
392	Fallen seed			
	germination	392		
393	Seed quality	393		
394	Seed insect			
	tolerance	394		
395	Seed disease			
	tolerance	395		
396	Seed size:			
	mg/100 seeds	396		: :

## Appendix 2. Dictionary of descriptors for forage germplasm collection and evaluation.

**General Information and Location of Collection Site.** The first 32 descriptors are considered essential and minimal information. Descriptors 1, 2, 3, 4, and 5 are included for administrative and identification purposes. They could be printed on the form by the collecting institution.

6. **Collector's number** is the number assigned by the collector in the field and should identify the collector as well as his number usually assigned in a consecutive manner. An example might be John Smith, sample number 1285 which would be recorded as JS1285, an alpha-numeric descriptor.
7. **Accession number** is a unique alpha-numeric descriptor which includes in the prefix an alphabetic code which identifies the country, followed by the number assigned by the national collection organization in the country. Ex. BRASIL27824. See Appendix 9 for the IS/GR alphabetical codes for the countries of the world.
8. **Other number** may be a number given to the same genotype by another collector (herbarium specimen, *Rhizobium* collection, etc.) or institution. This number may be useful for cross-referencing.
9. **Day, month, and year** indicates when collection was made.
10. **Genus** if known or provided later before keypunching.
11. **Species** if known or provided later before keypunching.
12. **Local name** is the name given by the local people, which may be very valuable in locating the species in future collection trips.
13. **Sample source** indicates whether the sample was collected in the wild or obtained from another institution or experimental station.

14. **Locality** should be located as precisely as possible as so many kilometers in a certain direction **from** or **to** the nearest village (15).
15. **Nearest village** goes with descriptor 14. For ease of relocation, collectors are urged to use place names as listed in the Times Atlas of the World (Comprehensive Edition).
16. and 17. **District/country and province/state** could be obtained from political maps or by local inquiry.
18. **Ethnic group** refers to the specific group of native people residing in the area where the sample was collected.
19. and 20. **Latitude and longitude** should be recorded by the collector at headquarters. Grid reference maps of a scale of 1:250,000 or larger are desirable when attempting to locate accurately the collection site.
21. **Donor's name** refers to the name of an institute or individual from whom the sample may have been obtained.
22. **Donor's number** is the collection number assigned by the donor.
23. **Donor's source** refers to the place where the donor obtained the sample.
24. **Plant collected** is the form of propagation of the material collected, whether seed or vegetative material and space is provided for the collector to specify.

**Natural Habitat and Area Vegetation.** This group of descriptors provides information on the macroenvironment and are indispensable for future study of environmental-agronomic interactions and the selection of adapted ecotypes of forage plants for particular niches in the environment. The first three (40-42) descriptors can best be recorded at headquarters whereas, the last five (43-47) can only be completed in the field by seeing the collection site.

40. **Rainfall** for the total year should be recorded.
41. **Rainfall seasonality** should be recorded as the season when precipitation occurs:
  - 1) all seasons (A)
  - 2) summer drought (B)
  - 3) winter drought (W)
  - 4) dry all season (D)

The number of dry months, if known, should be recorded.

42. **Altitude** may be obtained from topographic maps when they are available or from a well-calibrated altimeter.



43. **Topography** at collection site is described as:

- 1) swamp (S)
- 2) flood plain (F)
- 3) level (L)
- 4) undulating (U)
- 5) hilly (H)
- 6) mountainous (M)
- 7) other (O) space provided on collection form, if none of the first six apply.

44. **Vegetation type** is described as:

- 1) forest (F)
- 2) grassland (G)
- 3) desert (D)

45. **Local vegetation** name should be filled in if there is a unique local name available.  
Ex. - cerrado, llanos, thorn veld, sabanas, etc.

46. **Land use** may be described in one of eight categories:

- 1) recently disturbed area (D)
- 2) native pasture (N)
- 3) improved pasture (I)
- 4) cultivated (C)
- 5) roadside (R)
- 6) waterside (W)
- 7) settlement (S)
- 8) other (O)

Space is provided to write in other land use if none of the seven apply.

47. **Management** may be:

- 1) cutting (C)
- 2) cattle grazing (B)
- 3) sheep grazing (S)
- 4) goat grazing (G)
- 5) plowing (P)
- 6) irrigation (I)
- 7) burning (F)

Space is provided for other management if none of the seven apply.

#### **Descriptors for Specific Collection Site**

60. **Position in landscape** is described as:

- 1) low (L)
- 2) medium (M)
- 3) high (H)

These words refer to the position of the collection site in relation to the overall landscape. As an example, half way up a valley slope would be medium.

61. **Slope of the specific collection site is described as:**

- |                            |        |
|----------------------------|--------|
| 1) flat or almost flat (F) | 0-1°   |
| 2) gently sloping (G)      | 1-3°   |
| 3) sloping (SL)            | 3-7°   |
| 4) moderately sloping (MS) | 7-14°  |
| 5) steep (ST)              | 14-29° |
| 6) very steep (VSP)        | > 29°  |

If possible the slope should be described in quantitative terms determined by an Abney level or similar instrument.

62. **Slope facing** or aspect of a sloping site may be recorded as a sector, e.g., N, NE, E, SE, S, SW, W, NW by taking a reading parallel to the direction of, and facing outwards from, maximum slope. It may be assumed that slopes of less than 3° have no slope.

63. **Site ground cover** is estimated within the area approximately 1 m<sup>2</sup> surrounding the plant, although the area might be larger or smaller depending upon the size of the plant. The ground cover may be described as:

- |                    |           |
|--------------------|-----------|
| 1) bare (B)        |           |
| 2) very thin (VT)  | 0 - 19%   |
| 3) thin (T)        | 20 - 39%  |
| 4) moderate (M)    | 40 - 59%  |
| 5) heavy (H)       | 60 - 79%  |
| 6) very heavy (VH) | 80 - 100% |

64. **Degree plant shaded** is estimated according to the following categories and the sources of shade are listed under code 65:

- 1) no shade (N)
- 2) very slight (VS)
- 3) slight (S)
- 4) moderate (M)
- 5) heavy (H)
- 6) completely (C)

65. **Shaded by** refers to the source of shade indicated under code 64.

- 1) weeds (W)
- 2) grasses (G)
- 3) shrubs (S)
- 4) trees (T)
- 5) topography (H)

Topography for this purpose includes buildings, bridges, drains and other obstructions as well as cliffs and depressions.

66. **Target species** refer to the species being collected (10 and 11) and its life form is recorded in one category as follows:

- |              |           |
|--------------|-----------|
| 1) trees     | > 30 m    |
| 2) trees     | 10 - 30 m |
| 3) trees     | 5 - 10 m  |
| 4) shrubs    | 2 - 8 m   |
| 5) subshrubs | 0 - 2 m   |
| 6) herbs     | > 1 m     |
| 7) herbs     | 0 - 1 m   |

67. **Target species, growth habit** is described by one or more of the following descriptors:

- |                       |  |
|-----------------------|--|
| 1) prostrate (P)      | - lying flat on ground.  |
| 2) decumbent (D)      | - reclining or lying on ground with end ascending.                               |
| 3) erect (E)          | - nearly vertical.   |
| 4) vine (V)           | - a climbing vine with tentacles.  |
| 5) sarmentose (S)     | - producing slender prostrate branches or runners.                               |
| 6) liana (L)          | - a climbing vine without tentacles.   |
| 7) caespitose (C)     | - growing in clusters.   |
| 8) rhizomatous (R)    | - creeping stem below soil surface capable of producing new shoots at the nodes. |
| 9) stoloniferous (ST) | - creeping stem on soil surface capable of producing new shoots at the nodes.    |
| 10) rootstock (RS)    | - underground spreading root, morphologically root tissue.                       |

68. **Target species, rooting habit** may be of interest since there is a large variation in rooting habit between species and among ecotypes within species. The following categories are for all types of forage plants:
- 1) deep tap root (DT)
  - 2) shallow tap root (ST)
  - 3) shallow spreading (SS)
  - 4) deep spreading (DS)
  - 5) fibrous (F)
69. **Target species, stage of growth** refers to the stage of growth at time of collection.
- 1) vegetative (V)
  - 2) flowering (A)
  - 3) fruiting (F)
70. **Target species, longevity**
- 1) annual (A)
  - 2) biennial (B)
  - 3) perennial deciduous (PD)
  - 4) perennial evergreen (PE)
71. **Associated species 1** refers to the most frequent species growing in association with the target species. Either the botanical name (if known) or the local common name should be recorded.
72. The **life form** of the associated species 1 should be recorded as in descriptor 66 which defines the life forms.
73. and 74. The second most frequent associated species and its life form should be recorded as in 71 and 72.

#### **Descriptors of Soil Characteristics**

80. **Soil texture** is classified in the field in one of five categories.
- 1) sandy (S)
  - 2) loamy (L)
  - 3) clayey (C)
  - 4) organic (O)
  - 5) rocky (R)

81. **Soil surface color**

- 1) red (R)
- 2) yellow (Y)
- 3) brown (B)
- 4) gray (G)

82. **Soil drainage.** The overall concept of drainage is a broad one, embracing surface run-off, soil permeability, and internal soil drainage. The seven categories described in the U.S.D.A. Soil Survey Manual (1951) are reproduced here.

- 1) **Ponded** - none of the water added to the soil as precipitation or by flow from surrounding higher land escapes as run-off. The total amount of water that must be removed from ponded areas by movement through the soil or by evaporation usually is greater than the total rainfall. Ponding normally occurs in areas and may fluctuate seasonally.
- 2) **Poorly drained** - surface water flows away so slowly that free water lies on the surface for long periods. The water table is commonly at or near the surface during a considerable part of the year.
- 3) **Imperfectly drained** - water is removed from the soil slowly enough to keep it wet for significant periods but not all the time.
- 4) **Moderately well-drained** - surface water flows away at such a rate that a moderate proportion of the water enters the soil profile and free water lies in the surface for only short periods.
- 5) **Well-drained** - water is removed from the soil readily but not rapidly.
- 6) **Somewhat excessively drained** - a large proportion of the precipitation moves rapidly over the surface of the soil and a small part moves through the soil profile. Surface water runs off nearly as fast as it is added.
- 7) **Excessively drained** - a very large part of the water moves rapidly over the surface of the soil and a very small part goes through the profile. Surface water runs off as fast as it is added.

83. **Soil pH** - Since the method used to determine soil acidity influences the pH value obtained in the laboratory, the method used should be indicated.

- 1) 1:1 soil: water suspension (1)
- 2) 1:3 soil: water suspension (3)
- 3) 1:8 soil : water suspension (8)
- 4) 1:1 soil : 1 N KCl suspension (K)

84. **Soil salinity** - self explanatory
85. **Soil P** - self explanatory
86. **Soil Ca** - self explanatory
87. **Soil Mg** - self explanatory
88. **Soil K** - self explanatory
89. **Soil Al** - self explanatory
90. **Effective Cation Exchange Capacity** - self explanatory
91. **Al saturation** - self explanatory
92. **Other nutrients** - space is provided for the recording of two other nutrients together with amounts present in the soil in ppm or meq/100g of soil whichever is appropriate.

#### **Description of Insects and Diseases**

200. **Insects** - name family or genus if known and indicate type as follows:

- 1) sucking (S)
- 2) chewing (C)
- 3) boring (B)
- 4) not known (X).

201. **Insects - part of plant attacked:**

- 1) leaves (L)
- 2) stems (S)
- 3) roots (R)
- 4) inflorescence (I).

202. **Insects - plant tolerance:**

- 1) poor (P)
- 2) fair (F)
- 3) moderate (M)
- 4) good (G)
- 5) excellent (E).

250. **Diseases** - name the genus if known and indicate type of disease:

- 1) fungus (F)
- 2) bacteria (B)
- 3) virus (V)
- 4) nematodes (N)
- 5) mineral deficiency (MD)
- 6) mineral toxicity (MT)
- 7) not known (X).

251. **Diseases** - part of plant attacked:

- 1) leaves (L)
- 2) stems (S)
- 3) roots (R)
- 4) inflorescence (I).

252. **Diseases** - plant tolerance:

- 1) poor (P)
- 2) fair (F)
- 3) moderate (M)
- 4) good (G)
- 5) excellent (E).

#### **Description of Evaluation Site**

301. **Date received** by the evaluator.

302. **Accession** number assigned by the local evaluator.

303- 307. **Name and address** of individual and or institution supplying the planting material (seed, vegetative etc.).

308- 312. **Name and address of evaluator**

313. **Year** in which the accession is being evaluated.

314. Self explanatory

315. **Rainfall seasonality** is recorded as the season when precipitation occurs:

- 1) all seasons (A)
- 2) summer drought (S)
- 3) winter drought (W)
- 4) dry all seasons (D)

The number of dry months, if known, should be recorded.

316. Self explanatory
317. Self explanatory
318. Self explanatory
319. **Soil texture** is classified as follows:
- 1) sandy (S)
  - 2) loamy (L)
  - 3) clayey (C)
  - 4) organic (O)
  - 5) rocky (R)
320. **Soil surface color**
- 1) red (R)
  - 2) yellow (Y)
  - 3) brown (B)
  - 4) gray (G)
321. **Soil drainage** is described in seven categories in accordance with the U.S.D.A. Soil Survey Manual (1951): Soil Survey Manual (1951); for description see 82.
322. **Soil pH** - Since the method used to determine soil acidity influences the pH value obtained in the laboratory, the method used should be indicated:
- 1) 1:1 soil: water suspension (1)
  - 2) 1:3 soil: water suspension (3)
  - 3) 1:8 soil: water suspension (8)
  - 4) 1:1 soil: 1 N KCl suspension (K)
323. **Soil salinity** - self explanatory
324. **Soil P** - self explanatory
325. **Soil Ca** - self explanatory
326. **Soil Mg** - self explanatory
327. **Soil K** - self explanatory
328. **Soil Al** - self explanatory
329. **Effective Cation Exchange Capacity** - self explanatory
330. **Al saturation** - self explanatory



331. **Other nutrients** - space is provided for the recording of two other nutrients together with amounts present in the soil in ppm or meq/100g of soil, whichever is appropriate.
332. **Fertilizer applied** - the amount of fertilizer applied to the evaluation nursery is recorded as kilograms per hectare of each of the major elements.
333. **Lime applied** - calcic or dolomitic limestone should be indicated, together with the amount in kilograms per hectare of CaCO<sub>3</sub>-equivalent.

#### **Description of Planting Procedure**

340. **Planted in laboratory** - space is provided for method of planting, e.g., petri dish, media, seed treatment, etc. and the date when planted.
341. **Planted in glasshouse** - space is provided for the evaluator to record the planting medium or container plus the date of planting.
342. **Planted in field** - space is provided for the evaluator to record any unusual circumstance concerning the planting medium in the field plus the date of planting.
343. **Plot configuration** - refers to type of plot and the spacing and/or size of plot:

- |                     |                     |
|---------------------|---------------------|
| 1. single plant (S) | e.g., 2 meters      |
| 2. row (R)          | e.g., 10 plants/3 m |
| 3. plot (P)         | e.g., 1 x 4 m       |

344. **No. of replications** - self explanatory

345. **Seedling survival:**

- 1) poor (P)
- 2) fair (F)
- 3) moderate (M)
- 4) high (H)
- 5) very high (V).

346. **Seedling vigor:**

- 1) poor (P)
- 2) fair (F)
- 3) moderate (M)
- 4) high (H)
- 5) very high (V)

**347. Establishment success:**

- 1) poor (P)
- 2) fair (F)
- 3) moderate (M)
- 4) high (H)
- 5) very high (V).

**Description of Growth and Flowering**

**360. Herbarium specimen** - this is a herbarium specimen collected in the evaluation nursery. If the collection appears to be a mixture it may be advisable to acquire more than one specimen.

**361. Longevity:**

- 1) annual (A)
- 2) biennial (B)
- 3) perennial deciduous (D)
- 4) perennial evergreen (E).

**362. Life form** - the life forms are classified as:

- |              |           |
|--------------|-----------|
| 1) trees     | > 30 m    |
| 2) trees     | 10 - 30 m |
| 3) trees     | 5 - 10 m  |
| 4) shrubs    | 2 - 8 m   |
| 5) subshrubs | 0 - 2 m   |
| 6) herbs     | > 1 m     |
| 7) herbs     | 0 - 1 m   |

**363. Growth habit;** for description see 62.

**364. Flowering type:**

- 1) determinate (D)
- 2) indeterminate (I)
- 3) unknown (X)

**365. Rooting habit** - there is a large variation in rooting habit between species and among ecotypes within species. The type of root system may determine in a large measure the plant survival under a variety of stresses (frost, cold, drought, water-logging, etc.).

- 1) deep tap root (DT)
- 2) shallow tap root (ST)

- 3) shallow spreading (SS)
- 4) deep spreading (DS)
- 5) fibrous (F)

366. **Nodulation** - indicate part of root system where nodules occur:

- 1) crown area (C)
- 2) taproot (T)
- 3) lateral roots (L)

367. **Nodulation** - indicate whether there is evidence of the rhizobia being active:

- 1) active (A) -brown, pink or red when sectioned
- 2) inactive (N) -white or green when sectioned
- 3) absent (W)

368. **Regrowth mechanism** - indicate the part or parts of the plant from which new growth appears after defoliation:

- 1) basal or crown buds (B)
- 2) axillary buds (A)
- 3) leaf elongation (L)
- 4) stolons (S)
- 5) rhizomes (R)

Note: The descriptors 369 to 383 inclusive are characters which can be recorded over the growth period of the accession. In some environments and for some species this growth period may be for the entire 12 months of the year. Most of these descriptors can be estimated visually on a 1 to 5 rating scale with 1 being the least desirable expression of the descriptor and 5 the best expression of the descriptor. Space is provided in Appendix 1 for a rating to be made once each month on each descriptor. If the evaluator wishes to provide a more frequent rating then additional space would need to be provided.

369. **Regrowth capacity:**

- 1) poor (P)
- 2) fair (F)
- 3) moderate (M)
- 4) good (G)
- 5) excellent (E)

370. **Overall vigor** - ratings as above

371. **Leafiness** - ratings as above

372. **Deciduous** - check the months during which the plant is deciduous

373. **Frost tolerance:**

- 1) poor (P)
- 2) fair (F)
- 3) moderate (M)
- 4) good (G)
- 5) excellent (E)

374. **Cold tolerance** - ratings as above

375. **Drought tolerance** - ratings as above

376. **Water-logging tolerance** - ratings as above

377. **Flooding tolerance** - ratings as above

378. **Flowering** - indicate by months the abundance of flower production:

- 1) negligible (N)
- 2) few (F)
- 3) moderate (M)
- 4) many (A)
- 5) maximum (X)

379. **Yield estimate** - visual estimate (rating) of yield of **introduction** by months:

- 1) poor (P)
- 2) fair (F)
- 3) moderate (M)
- 4) good (G)
- 5) excellent (E).

380. **Yield estimate** - visual estimate (rating) of yield of **standard** species or cultivar by months:

- 1) poor (P)
- 2) fair (F)
- 3) moderate (M)
- 4) good (G)
- 5) excellent (E).

381. **Plant spread** - range of spread of **introduction** by vegetative means or seed recorded by months:

- 1) poor (P)
- 2) fair (F)
- 3) moderate (M)
- 4) good (G)
- 5) excellent (E).

382. **Plant spread** - range of spread of **standard** species or cultivar by months:

- 1) poor (P)
- 2) fair (F)
- 3) moderate (M)
- 4) good (G)
- 5) excellent (E)

383. **Crude protein, %** - space is provided for the recording of crude protein percent on six different occasions.

384. **Digestibility, IVOMD, %** - space is provided for the recording of IVOMD % on six different occasions.

#### **Description of Seeding Characteristics**

390. **Seed production** - a visual rating of seed production by months so that the period of maximum seed production can be identified:

- 1) poor (P)
- 2) fair (F)
- 3) moderate (M)
- 4) good (G)
- 5) maximum (X).

391. **Seed shattering resistance** - visual estimate of seed shattering is made on a rating scale:

- 1) poor (P)
- 2) fair (F)
- 3) moderate (M)
- 4) good (G)
- 5) excellent (E).

392. **Fallen seed germination** - visual estimate of germinating seedlings:

- 1) negligible (N)
- 2) few (F)
- 3) moderate (M)
- 4) many (A)
- 5) abundant (X).

393. **Seed quality** - visual appearance:

- 1) poor (P)
- 2) fair (F)
- 3) moderate (M)
- 4) good (G)
- 5) excellent (E)

394. **Seed insect tolerance** - visual estimate of resistance to insect damage:

- 1) poor (P)
- 2) fair (F)
- 3) moderate (M)
- 4) good (G)
- 5) excellent (E).

395. **Seed disease tolerance** - visual estimate of resistance to disease damage:

- 1) poor (P)
- 2) fair (F)
- 3) moderate (M)
- 4) good (G)
- 5) excellent (E).

396. **Seed size: mg/100 seeds** - self explanatory.

### **Appendix 3. Procedure for sending nodule samples to CIAT.**

Four weeks in advance of dispatch of samples, forward the following information to CIAT:

- Name of person sending the sample
- Name of his institute or organization
- Address of his institute or organization
- Locality of origin of samples
- Means of dispatch (air mail)
- Approximate number of samples

CIAT will forward a permit in the form of a self-adhesive label, which must accompany the samples.

The permit is not valid unless the nodule samples are contained in glass vessels with air-tight lids and the outside of all tubes are sterilized with mercuric chloride solution (external labeling must withstand this treatment). All materials used to pack the glass vessels must be new, not re-used.

It is essential that the collector contact the collaborating laboratory, if other than CIAT, for their specific procedures.

### **Appendix 4. List of laboratories which will isolate strains of *Rhizobium* on behalf of collector.**

#### **ARGENTINA**

Elizabeth G. de Olivero  
Unidad Simbiosis I.N.T.A.  
Casilla de Correo No. 25  
Castelar, Provincia de Buenos Aires

#### **AUSTRALIA**

Richard A. Date  
Division Tropical Crops and Pastures  
C.S.I.R.O. Cunningham Laboratory  
Mill Road, St. Lucia, Queensland 4067

## **BRAZIL**

Avilio A. Franco  
EMBRAPA-SNLCS-PFN, Km. 47  
23460, Seropedica  
Rio de Janeiro

Eli Sidney Lopez  
Instituto Agronomico de Campinas  
Av. Barao de Itapura 1481  
C.P. 28 Campinas  
Sao Paulo

Milton Vargas  
EMBRAPA, Centro de Pesquisa  
Agropecuaria dos Cerrados  
BR-20, Km. 18, Brasilia  
Planaltina, 70100

J.R. Jardim Freire  
Facultad de Agronomia  
Universidad Federal do Rio Grande do Sul  
Caixa Postal 776  
Porto Alegre

## **COLOMBIA**

Microbiology Section  
Tropical Pastures Program  
Centro Internacional de Agricultura Tropical  
(CIAT)  
Apartado Aéreo 67-13  
Cali

## **CHILE**

Luis Bernardo Longeri  
Departamento de Microbiologia  
Universidad de Concepción  
Casilla de Correo 272  
Concepción

## **KENYA**

S.O. Keya  
MIRCEN Director and Chairman  
Department of Soil Science  
University of Nairobi  
P.O. Box 30197  
Nairobi

## **MEXICO**

María Valdés  
Departamento de Microbiología  
Instituto Politécnico Nacional  
Apartado Postal 4-870  
Mexico, D.F.

Antonio Moreno Quiroz  
Banco de México  
Apartado Postal No. 27  
Jiutepec  
Morelos

## **PANAMA**

Blanca C. de Hernández  
Escuela de Microbiología  
Facultad de Biología  
Universidad de Panamá  
Panamá

## **PERU**

Jose Carrion Gómez  
I.V.I.T.A.  
Universidad Nacional Mayor  
de San Marcos  
Museo Historia Natural  
Avenida Arenales 1256  
Apartado 1256  
Lima

## **SOUTH AFRICA**

Ben W. Strijdom  
Plant Protection Research Institute  
Private Bag 134  
Pretoria

## **UNITED STATES OF AMERICA**

Deane F. Weber or Harold Keyser  
CCNF - Rhizobium Collection  
USDA-BARC  
Beltsville, MD 20705

Victor Reyes  
University of Hawaii-Niftal  
P.O. Box "O"  
Paia, HI 96779

**VENEZUELA**

Ivan Casas  
Apartado 1664  
Maracaibo, Estado Zulia

**Appendix 5. Sample form for seed export in India.**

No. \_\_\_\_\_ Dated \_\_\_\_\_

**ADVANCE INTIMATION OF EXPORT OF SEED SAMPLES**

To  
The Project Director  
(Address)

Sir:

The following consignment has been dispatched separately to you for plant quarantine inspection.

1. Name of Scientist:  
Local Address:
2.
  - i) Crop (with botanical name):
  - ii) No. of boxes/bags/cartons:
  - iii) Distinguishing marks:
3. Weight:
4. Mode of dispatch: Air cargo/Post parcel/Accompanied  
baggage/Unaccompanied baggage
5. Particulars of phytosanitary certificate:
6. General health, pest incidence/intensity on crop at time of seed collection:
7. Date (s) of collection:
8. Date of dispatch:
9. Remarks, if any:

Date: \_\_\_\_\_ Signature: \_\_\_\_\_

cc: \_\_\_\_\_ (Name)

- Note:
1. Please fill in separate form for each crop/each country.
  2. Scientists importing germplasm as accompanied/unaccompanied baggage may fill in the form immediately after arrival.



**Appendix 6.** List of countries and the authorities issuing phytosanitary certificates.

**ARGENTINA**

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

**AUSTRALIA**

Assistant Director-General  
(Plant Quarantine)  
Dept. of Health  
Canberra, A.C.T.

**BOLIVIA**

Jefe  
Division Nacional de Sanidad  
Vegetal  
Ministerio de Agricultura  
Avda. Camacho No. 1471, La Paz

**BRAZIL**

Director  
Secretaria de Defensa  
Sanitaria Vegetal-MA  
Ed. Venancio 2000, 3º andar  
70,000-Brasilia-D.F.

**CHILE**

The Director  
Departamento de Defensa Agrícola  
Ministerio de Agricultura  
Casilla No. 4647, Santiago

**COLOMBIA**

The Director  
Servicio de Sanidad Vegetal  
Ministerio de Agricultura  
Calle 37 # 8-43, piso 8  
Bogotá, D.E.

**Appendices**

**COSTA RICA**

Jefe  
Dept. de Cuarentena y Registro  
Ministerio de Agri. y Ganadería  
San José

**CUBA**

The Director  
Departamento de Cuarentena  
Vegetal  
Dirección Nacional de Sani-  
dad Vegetal, INRA  
La Habana

**CURACAO**

Director of Agriculture  
Plantentium Cas-cora  
Willemstand

**DOMINICAN REPUBLIC**

The Director  
Dept. de Sanidad Vegetal  
Secretaría de Estado de Agri.  
Santo Domingo, D.N.

**ECUADOR**

Jefe  
Servicio de Sanidad Vegetal  
Ministerio de Agricultura y  
Ganadería,  
Quito

**EL SALVADOR**

Jefe Departamento de Cuarentena  
Agropecuaria  
Ministerio de Agri. y Ganadería  
San Salvador

**GUATEMALA**

Jefe  
Departamento de Sanidad  
Vegetal y Cuarentena  
Ministerio de Agricultura  
12 Avenida Sur y 19 Calle Oeste  
Guatemala

**GUAYANA**

Chief Agricultural Officer  
Ministry of Agri. & Natural  
Resources  
Georgetown

**HAITI**

Chef  
Service de Quarantine  
Dept. de L'agriculture  
Damien, Port-au-Prince

**HONDURAS**

Jefe  
Dept. de Sanidad Vegetal  
Ministerio de Recursos Naturales  
Tegucigalpa, D.C.

**JAMAICA**

Chief Plant Protection Officer  
Plant Protection Division  
Ministry of Agri. & Fisheries  
P.O. Box 480, Hope  
Kingston 6

**MEXICO**

Jefe  
Dept. de Aplicación  
Cuarentenaria  
Dirección General de  
Sanidad Vegetal  
Secretaría de Agricultura  
y Ganadería  
Balderas 94, Mexico 1, D.F.

**90****NICARAGUA**

Jefe  
Dept. de Sanidad Vegetal  
Ministerio de Agri. y Ganadería  
Managua, D.N.

**PANAMA**

Jefe  
Sección de Cuarentena  
Agropecuaria  
Dept. de Investigación  
Agrícola  
Ministerio de Agricultura  
Comercio e Industrias  
Panamá, R. de P.

**PARAGUAY**

Dirección de Defensa  
Agrícola  
Asunción

**PERU**

Jefe  
División de Defensa Agrícola  
Ministerio de Agricultura  
Lima

**PUERTO RICO**

Inspector-in-charge  
Agri. Quarantine Inspection  
Division  
U.S. Dept. of Agriculture  
P.O. Box 3386  
San Juan, 00904

**TRINIDAD & TOBAGO**

Technical Officer (Res.)  
Ministry of Agriculture  
Lands & Fisheries  
Centeno

**Tropical Forage Germplasm Resources**

## UNITED STATES OF AMERICA

The Director  
Agri. Quarantine Inspection  
Division  
Agri. Research Service  
Dept. of Agriculture  
Federal Center Building  
Washington, D.C. 20250

## VENEZUELA

Jefe  
División de Sanidad Vegetal  
M.A.C.  
Ministerio de Agri. y Cría  
Torre Norte - Centro Simón  
Bolívar  
Piso 14, Caracas

## URUGUAY

The Director  
Servicio de Lucha Contra  
las Plagas  
Ministerio de Ganadería y Agric.  
Maldonado 1276  
Montevideo

### **Appendix 7. Main recommendations and conclusions of the International Board for Plant Genetic Resources (IBPGR) Working Group on Engineering, Design and Cost of Long-term Seed Storage Facilities\*.**

1. The recommendations apply to the storage of orthodox seeds.
2. Acquisitions should be stored in sealed containers at about 5% moisture content in a cold room held at -20°C. The temperature could be relaxed to -10°C in special cases.
3. Suitable alternative sealed containers, with certain reservations, include glass jars, cans and laminated foil packets.
4. In general there is no need for special provision to control the relative humidity in cold storage rooms.
5. Seed drying needs to be carefully controlled; several alternative systems would be satisfactory.
6. Moisture determinations and periodic routine germination tests should be based on International Seed Testing Association (ISTA) rules, with certain modifications, in order to minimize the amount of seed used for these purposes.

---

\* The full report is available from the IBPGR Secretariat, Rome, Italy.

7. Ideal sizes for individual accessions will depend not only on the requirements for routine testing but also mainly on the genetic heterogeneity of the accessions. In base collections the ideal is likely to be within the range 3,000-12,000 seeds.
8. Careful consideration should be given to the siting of a cold storage facility.
9. Prefabricated panel construction of the cold room is recommended. The entrance door should be heated and an air lock provided. Precautions should be taken to prevent frost heave in the floor.
10. The use of mobile shelving to house the accessions in the cold store is economic and considered desirable.
11. Refrigeration should be carried out using direct or indirect vapor compression systems, using conventional refrigerants and air-cooled condensers. There should be adequate recirculation of the chilled air within the store (5-10 changes/hour).
12. Safety precautions which should be adopted include the provision of two refrigeration units and a standby generator, incorporation of various visible and audible warning devices should the refrigeration plant fail, and devices to ensure that nobody is accidentally locked in the store. Additional precautions should be taken where earth tremors are common.
13. It is recommended that specifications of cold rooms should be based on American Society of Heating and Refrigeration Engineers (ASHRE) standards.
14. Ancillary rooms and equipment need to be provided for the operation of a long-term seed storage facility. Provision is required for seed drying, cleaning, testing and packaging, and for records and services.
15. It is judged that the requirements of many banks will be met by cold rooms within the range 85-200 m<sup>3</sup> (approximately 22,000-60,000 acquisitions), although this report has covered the range up to approximately 280 m<sup>3</sup> (90,000 acquisitions) to allow for special circumstances.
16. The capital cost of cold storage rooms, including shelving, is likely to vary according to the size of the store from \$0.80 to \$1.20 per acquisition stored. The capital cost of providing a complete seed storage bank, with minimum satisfactory ancillary facilities, is likely to vary from \$1.89 to \$10.74 per acquisition, according to the size and location of the bank.

Appendix 8. Recommended conditions for germination tests of forage legumes and grasses.

Species	Medium*	Temperature ° C	Light	Counting period (days)		Additional Treatments
				First	Final	
<i>Alysicarpus vaginalis</i>	BP	35	-	4	21	Pierce seedcoat at 21 days, grows to 35 days
<i>Brachiaria decumbens</i>	TP	20-35	+	4	21	Pre-dry, nitrate
<i>Centrosema pubescens</i>	TP	20-35	-	4	10	Nitrate; pre-chill at 10°C 7 days
<i>Cynodon dactylon</i>	TP	20-35; 20-30	+	7	21	
<i>Desmodium intortum</i>	TP	20-30	-	4	10	
<i>Macroptilium atropurpureum</i>	TP	25	-	4	10	
<i>Medicago sativa</i>	TP, BP	20	-	4	10	Pre-chill
<i>Panicum maximum</i>	TP	20-35; 15-35	+	10	28	Nitrate
<i>Paspalum notatum</i>	TP	30-35	-	3	21	Nitrate; remove glumes, scratch caryopsis
<i>Stylosanthes guianensis</i>	TP	20-35; 10-35; 20-30	-	4	10	
<i>Stylosanthes humilis</i>	TP	25	-	4	10	

\* Abbreviations: BP = between paper; TP = on top of paper; nitrate = moisten the germination medium with a 0.2% solution of KNO<sub>3</sub> (i.e. 2 g of the compound dissolved in 1 liter of water). Subsequently moisten the medium with water only.

**Appendix 9. Alphabetic codes for countries\*.**

<b>Code</b>	<b>Country</b>	<b>Code</b>	<b>Country</b>
AFGH	Afghanistan	GERM E	Germany - East
ALBNA	Albania	GHANA	Ghana
ALGER	Algeria	GREECE	Greece
ANDOR	Andorra	GUATM	Guatemala
ARGNT	Argentina	GUINEA	Guinea
AUSTL	Australia	GUYANA	Guyana
AUSRI	Austria	HAITI	Haiti
BANGL	Bangladesh	HONDR	Honduras
BARBD	Barbados	HUNGARY	Hungary
BELGM	Belgium	ICELD	Iceland
BHUTN	Bhutan	INDIA	India
BOLIV	Bolivia	INDON	Indonesia
BOTSW	Botswana	IRAN	Iran
BRAZL	Brazil	IRAQ	Iraq
BULGR	Bulgaria	IRELD	Ireland
BURMA	Burma	ISRAEL	Israel
BURUN	Burundi	ITALY	Italy
CAMBD	Cambodia	IVO CO	Ivory Coast
CAMER	Cameroon	JAMAI	Jamaica
CANAD	Canada	JAPAN	Japan
CAFR	Central African Republic	JORDAN	Jordan
CHAD	Chad	KENYA	Kenya
CHILE	Chile	KOREA N	Korea - North
CHINA C	China (Communist)	KOREA S	Korea - South
CHINA T	China (Taiwan)	KUWAIT	Kuwait
COLOMB	Colombia	LAOS	Laos
CONGO	Congo	LEBN	Lebanon
CO RIC	Costa Rica	LESOTH	Lesotho
CUBA	Cuba	LIBER	Liberia
CYPRS	Cyprus	LIBYA	Libya
CZECH	Czechoslovak Socialist Republic	LICHT	Liechtenstein
DAHOM	Dahomey	LUXMB	Luxembourg
DENMK	Denmark	MALAG	Malagasy Republic
DOM R	Dominican Republic	MALAWI	Malawi
ECUAD	Ecuador	MALAY	Malaysia
EL SAL	El Salvador	MALDV	Maldives Islands
ETHOP	Ethiopia	MALI	Mali
FINLD	Finland	MALTA	Malta
FRANCE	France	MAURI	Mauritania
GABON	Gabon Republic	MEXICO	Mexico
GAMBIA	Gambia	MONACO	Monaco
GERM W	Germany -West	MONGO	Mongolia, People's Republic of

\* Developed by IS/GR for international germplasm information exchange.

<b>Code</b>	<b>Country</b>	<b>Code</b>	<b>Country</b>
MOROC	Morocco	SRILK	Sri Lanka
MUSCAT	Muscat	SUDAN	Sudan
NEPAL	Nepal	SWEDEN	Sweden
NETHR	Netherlands	SWITZ	Switzerland
NEWZL	New Zealand	SYRIA	Syrian Arab Republic
NICAR	Nicaragua	USSR	Union of Soviet Socialist Republics
NIGER	Niger	TANZ	Tanzania
NIGIA	Nigeria	THAI	Thailand
NORWAY	Norway	TOGO	Togo
OMAN	Oman	TRINI	Trinidad
PAKST	Pakistan	TOBAGO	Tobago
PANAMA	Panama	TUNIS	Tunisia
PARAG	Paraguay	TURKEY	Turkey
PERU	Peru	UGANDA	Uganda
PHILP	Philippines	UAR	United Arab Republic
POLAND	Poland	UK	United Kingdom of Great Britain
PORTG	Portugal	USA	United States of America
RHOD	Rhodesia	UPVOLT	Upper Volta
RUMAN	Rumania	URUG	Uruguay
RWANDA	Rwanda	VATIC	Vatican City
SAMOA	Samoa	VENEZ	Venezuela
SAN MAR	San Marino	N VIETN	North Vietnam
S ARAB	Saudi Arabia	S VIETN	South Vietnam
SENGL	Senegal	YEMEN	Yemen
S LEON	Sierra Leone	YUGOS	Yugoslavia
SINGP	Singapore	ZAMBI	Zambia
SOMAL	Somalia Republic		
S AFR	South Africa		
SPAIN	Spain		

Personnel of the CIAT Communication Support Unit  
cooperating to produce this manual:

Editorial assistance :     Beatriz Davis  
Text composition :       Orfa Vásquez  
                                  Janeth Loaiza  
                                  Yolanda de González  
Graphic arts :            Libardo Didier González  
Photomechanics :        Luis E. Escobar



Apartado Aéreo 13283 - Bogotá - Colombia

MADE IN COLOMBIA