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FOREST PALMS AS TROPICAL CROPS
A REPORT TO U.S. AGENCY FOR INTERNATIONAL DEVELOPMENT

FINAL REPORT

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EXECUTIVE SUMMARY

This project involved the collaboration of Brazilian and Peruvian scientists, working with U.S. counterparts at Colorado State University, Fairchild Tropical Gardens and The New York Botanical Garden. The objectives included: collect palm genetic material in the Amazon region; develop and improve shoot proliferation techniques for palm propagation and cryobiological storage of palm germplasm; experimentally determine the role of mycorrhizal association in the growth rate and survival of the palm models; continue the NYBG training program for young LDC scientists in economic botany techniques; and, publish a document on the ongoing research program on forest palms as tropical resources. The model organisms selected included the palm genera *Bactris, Jessenia, Oenocarpus* and *Orbignya*, and we were able to build on existing studies with these plants. All of the objectives were accomplished. Two palm germplasm banks were established in Brazil, in Teresina PI, and Bacabal MA. A workshop was held (3/1986) on babassu palm research activities, which was attended by AID representative Dr. Carl Gallegos.

INTRODUCTION TO PROJECT

Introduction and Objectives

Amazonian palms of the genera *Jessenia*, *Oenocarpus*, and *Orbignya* are potential oilseed and biomass crops that boast established markets in their native areas. *Jessenia bataua* (Mart.) Burret, for instance, is an understory palm native to the northern half of South America. The fruit is collected throughout its natural range, and is eaten and processed for the production of edible oil. The oil content is as high as 50% of the mesocarp, and is similar in composition to olive oil (Balick 1984). *J. bataua*, which grows naturally in some of the poorest soils to be found anywhere, is an example of a potential crop for agriculturally marginal lands in the tropics. In spite of the desirable qualities of the fruit, virtually all commercial production comes from wild-grown trees.

Any large-scale use of these palms as crops will require the development of favorable and consistent genetics and practical methodology for the production of seedlings and the maintenance of field plantings. We perceive as first priorities the selection and preservation of the best genetic characteristics from the wide-range of wild types, the development of methods for preserving wild-collected material, and elaboration of techniques for the reliable production and outplanting of quality seedlings. During earlier work one of us (St. John) found that palm and other seedlings in Brazilian nurseries commonly lacked mycorrhizae. These symbiotic root-fungus associations are a normal part of the nutrient-acquisition of wild-grown palms (St. John 1987) and have

been found helpful and even indispensable in nursery culture of other kinds of plants. The reasons for their absence in the nursery, and the development of methods to allow their normal expression, has been the primary research focus of this project. Finally, the findings have to be available in the tropical countries where the palms can be grown, thus it has been considered essential to involve host country collaborators in the research and to train them to use the findings and to continue the research.

The project, which operated in both North and South America beginning in 1984, had the following overall objectives, as expressed in the original proposal:

1. Collect suitable genetic material in the Amazon region.
2. Develop and improve shoot proliferation techniques for palm propagation and cryobiological storage of palm germplasm.
3. Experimentally determine the role of mycorrhizal association in the growth rate and survival of these palm models.
4. Develop nursery procedures that allow establishment and maintenance of the mycorrhizal association.
5. Continue the existing NYBG program for training young LDC scientists in economic botany techniques, both in laboratory and through field exploration.
6. Publish a multi-lingual document on the proposed and ongoing US AID-funded research on forest palms as tropical resources.

The experimental work was divided between institutions to optimize use of available facilities and personnel. Collection of germplasm was the charge of The New York Botanical Garden (NYBG). Maintenance of germplasm was assigned to our two field sites and to the cooperative agreement between NYBG and CENARGEN in Brasilia. Investigation of in vitro methods was carried out by a commercial plant laboratory under a subcontract. Mycorrhizal investigations were divided between the Brazilian and Peruvian collaborators under consultation with St. John. Certain greenhouse and growth chamber experiments were established in Colorado and California by St. John. The response of palm species to mycorrhizal inoculation over a range of fertility levels was a large and complex experiment that required the infrastructure of the modern and well-staffed research station of CEPLAC in Bahia, Brazil. An experiment on the presence of inoculum in nursery soil was also carried out there. Work on performance of inoculated plants upon outplanting, and on the failure of VAM in the nursery was carried out at Yurimaguas, Peru. Inoculation and outplanting experiments were allocated to both stations. Several ancillary projects, some of them funded by other sources, were under way at each station. These included inoculation effects on other crops and screening and multiplication of promising VAM fungi. St. John served in an advisory capacity on some of the projects, although one is reported in detail here.

The project will first be described as a unit, by objectives, to clarify the relationship between the various experiments. The work at each field site will then be treated briefly in the context

of particular strengths and problems. Finally, the overall lessons and recommendations that have emerged from the work will be set forth in a conclusion section.

PROJECT RESEARCH AND RESULTS

Collection of Genetic Material

Wild genotypes of the three genera of primary concern, *Jessenia, Oenocarpus,* and *Orbignya* have been collected by Balick and collaborators in various South American expeditions since 1984. Some of the expeditions have been funded by this project and others have been separate, providing valuable input at little cost to the project.

In Table 1 is a summary of germplasm material collected for use by the mycorrhizal and tissue culture studies. This material was collected in a variety of habitats through the Amazon region. In most cases the collections emphasized production and other desirable traits. Other information on germplasm collection is to be found in the various appendices previously submitted with the progress reports. These are available upon request.

TABLE 1

GERMPLASM USED IN MYCORRHIZAL EXPERIMENTS

SPECIES	ACCESSION NUMBER	LOCATION
J. bataua	Rury 314	Peru
Orbignya sp.	Balick 1432	Bolivia
B. gasipaes	S.N.	Colombia
J. bataua	Balick 1647	Colombia
J. bataua	Balick 1647	Colombia
J. bataua	S.N.	Colombia
Euterpe oleracea	Balick 1660	Colombia
J. bataua	Forero 110	Colombia
J. bataua	Forero 111	Colombia

Table 2 shows soil properties of 14 samples from germplasm collection sites. These soils are of low pH and very low in phosphorus. Other properties vary somewhat. This emphasizes the remarkable ability of these palms to grow and produce in some of the worst soils anywhere.

TABLE 2
SOIL PROPERTIES FROM A NATIVE HABITAT OF TROPICAL AMERICAN PALMS; BOLIVIAN AND MEXICAN SITES

Sample	O.M. %	pH	P	K	Mg	Ca	Mn	Fe	Al	Zn
					PPM in soil					
#1	5.4	5.4	1.5	315	345	500	20	510	29	0.5
#2	ISA	5.3	4.0	145	325	1450	43	<2.5	16	0.25
#3	2.1	5.9	4.5	245	200	350	9.0	<2.5	10	0.25
#4	0.4	5.3	0.5	210	110	250	5.5	<2.5	30	0.25
#5	3.4	6.2	1.5	205	145	1050	16	<2.5	<5.0	<0.25
#6	3.8	6.3	3.5	55	185	1250	13	<2.5	<5.0	<0.25
#7	2.4	4.9	1.0	50	25	350	30	8.5	42	<0.25
#8	2.9	5.9	2.5	230	125	400	14	<2.5	7.5	0.25
#9	5.5	5.2	5.5	290	190	600	20	900	17	0.5
#10	ISA	7.9	5.5	290	925	53800	9.0	3.0	<5.0	<0.25
#11	4.5	8.0	2.0	95	390	53800	6.5	3.5	5.0	<0.25
#12	3.9	8.0	1.5	110	350	53800	6.5	3.0	<5.0	<0.25
#13	1.8	6.6	10	110	145	900	9.5	3.0	<5.0	1.5
#14	2.3	6.5	12	120	170	1000	10	<2.5	<5.0	1.5

#1, #2, #9: San Joaquin, Bolivia
#3 - #8: Ascencion, Bolivia
#10 - 14: Mexican Collections

The first priority in preserving collected germplasm has been the establishment of "germplasm banks" at our tropical research sites, where live plants are maintained for study of desirable traits and as a source of seed and material for in vitro procedures. We consider this work to have been successful and we are pleased to have appropriate media and in vitro techniques now available as a result of project efforts.

Some of the neutral variability among wild populations of *Jessenia bataua* is illustrated in color photo 1. Seed from diverse sources, including The New York Botanical Garden collections, was used in plantings of an agroforestry project near Una, Bahia, Brazil. Field personnel inform us that the size differences are a recent development. The growth rate of these plants has been slow in comparison with cultivated species of *Euterpe* and *Bactris*, but the several *J. bataua* plants visible in the photograph illustrate the striking variability in growth rate. According to station personnel, most of the differences expressed themselves only after an initial two or more years of rather uniform and very slow growth. The challenge now is to find the most favorable traits among wild plants, not only in growth rate, but in production and quality of fruit, so selection of usable economic material can begin.

SOMATIC EMBRYOGENESIS AND CRYOPRESERVATION

This work was carried out on sub-contract to Twyford Laboratories by Kenneth L. Giles (present affiliation Vipont Research Laboratories, Inc., Canada).

Introduction

Little work on somatic embryogenesis and cryopreservation has been done with palms, and the small amount to date has been done with three established commercial species: *Elaeis guineensis*, *Cocos nucifera* and *Phoenix dactylifera*. Thus we were fortunate to be able to enlist the services of a laboratory that has done some of the limited work with other palm species.

Work initiated at Twyford with forest palms included:

Electron microscopy of *O. speciosa* seed

Somatic embryogenesis of *O. speciosa* from intact and half embryos

X-ray examination of *O. speciosa* seed

Cryopreservation of excised embryos of *O. speciosa*

Cryopreservation of whole seed of *J. bataua*

Somtic Embryogenesis

Somatic embryogenesis, which refers to the production of functional plant embryos from tissues not normally destined to serve a reproductive function, is the most promising way to rapidly produce high quality plants once elite plants have been located in the field or produced by conventional genetic

techniques. Because these forest species have long generation times, and have not yet been subject to any efforts at genetic improvement, rapid "cloning" of any high quality individuals, such as those at Una with the highest growth rates, will be an invaluable tool.

Three important stages are involved in the use of tissue culture:

1. The establishment of sterile in vitro cultures;
2. The development and multiplication of somatic embryos;
3. The establishment of plantlets in soil.

The work with forest palms addressed the first two stages.

The Twyford work was carried out with *Orbignya speciosa*. Embryo cultures were attempted from surface-sterilized seed, which was then opened to extract the embryo. The embryos were plated on a sterile growth medium containing salts and hormones. After three weeks there was callus production on both intact and half embryos. By the sixth week, the callus was suitable for sub-culturing, and subsequently produced somatic embryos. No plant was carried through to the final step of establishment in soil, which according to Twyford would have required resources that were not available. The medium successfully used to callus induction is shown in Table 3.

Table 3. Proliferation Medium for *O. speciosa*

Murashige and Skoog salts

100 mg/L2, 4-D

2.5 mg/L 2iP

3 g/L activated charcoal

The successes that have been reported with commercial palm species include in vitro production of plantlets. Transfer of plantlets to independent growth in soil is difficult because of the change in humidity and the presence of fungal pathogens. A series of slow steps is required, during which a strong cuticle and root system is developed. This stage was not attempted with forest palms because of limited resources and limited material.

Cryopreservation

Storage of seeds of tropical forest trees is often impractical because of short viability. Cryopreservation, the storage of sterile tissues in a frozen condition, is a potential means of accumulating important germplasm. Methodology must be elaborated for each species and Twyford laboratory carried out preliminary investigations in this regard. The Twyford work on cryopreservation, like that on somatic embryogenesis, was carried out with *O. speciosa*.

One of the cryopreservation methods tested on *O. speciosa* was freezing the entire seed in a cryoprotectant. To judge the

success of the method, it must be known in advance whether the seeds are viable. For this reason, Twyford worked out an X-ray procedure for *O. speciosa* that allowed examination of the embryo before freezing. Whole-seed cryopreservation was attempted with and without the cryoprotectant D. M. S. O. The temperature was lowered to -51°C over a period of 10 hours. One of the five seeds available for the test produced a viable embryo.

Successful cryopreservation depends on freezing the tissues in a way that prevents the formation of ice crystals in the cells. This can be accomplished by freezing so rapidly that crystals do not have time to form, or by removing water from the cells. Different species have different susceptibility to dehydration and a range of procedures must be attempted if a successful method is to be found. Excised embryos of *O. speciosa* were treated with two cryoprotectants and frozen at two cooling rates. They were then thawed, sterilized, and plated on recovery medium. Contamination of the embryos was a major problem, as it often is with other palms, and a rather severe sterilization procedure was adopted in consequence. None of the excised embryos survived cryopreservation attempts.

Conclusions

The successes of Twyford with date and other palms has offered starting points for methodology with the forest palms. The preliminary work with *O. speciosa* is encouraging, especially with regard to somatic embryogenesis. It demonstrates the potential for in vitro propagation of elite individual plants, which can save years of research time in domestication of the forest palms. The additional step of establishment of plantlets in soil remains to be attempted. While difficult in its own right, the problems are perhaps somewhat more routine and less likely to differ markedly from established procedures than are the earlier steps, which have now been successfully completed.

Cryopreservation has been more elusive than somatic embryogenesis, but success was achieved with one of the few specimens available for the trials. Thus it has been demonstrated that the objective is attainable, even though further work will be required to optimize the methodology.

Subsequent to the work with Twyford, we established a collaborative research program on cryopreservation of palm seeds with The Royal Botanic Gardens, Kew. We expect that at least one research paper will be published as a result of this collaboration.

The Role of Mycorrhizal Association in Growth Rate and Survival

The symbiotic association of roots and fungi known as mycorrhiza is now widely recognized as critical in the phosphorus

nutrition of both wild and cultivated plants. This is especially true in crops that must grow without major inputs of fertilizer, as is the case with these forest palms. Mycorrhizal symbioses are classified into several distinct types, each of which involves a different kind of fungus and host plant. The most widespread type, found in palms and most other plants, are vesiculur-arbuscular mycorrhizae (VAM). Table 4 summarizes the occurrence of VAM in palms on the basis of field work for this project and various published and unpublished reports for comparison.

Table 4. Mycorrhizal Status of Palm Species

Species	VAM	Origin	Authority
Areca catechu	-	arboretum	Alwis & Abeynayake, 19
Arecastrum romanzoffianum	+	field	Hood, 1964
Arenga engeleri	+	field	Assai, 1934; Maeda, 19
Bactrus gasipaes	+	field	Janos, 1977
Bactris gasipaes	+	greenhouse	This project
Calamus sp.	+	field	Janse, 1897
Caryota urens	+	arboretum	Alwis & Abeynayake, 19
Chamaerops humilis	+	greenhouse	Kryuger, 1970
Cocos nucifera	+	field	Sampson, 1923
Cocos nucifera	+	field	Johnston, 1949
Cocos nucifera	+	field	Lily, 1975
Cocos nucifera	-	nursery	This project
Elais guianensis	+	field	St. John, unpub.
Euterpe oleracea	+	field	This project
Euterpe oleracea	+	greenhouse	This project
Euterpe edulis	+	field	This project
Jessenia bataua	-	field	This project
Jessenia bataua	+	greenhouse	This project
Livingstonia chinensis	+	field	Asai, 1934; Maeda, 1954
Oenocarpus bacaba	+	field	St. John, 1980
Phoenix dactylifera	-,+	field	Trappe, 1981
Phoenix roebelenii	-	greenhouse	Stanczak-Boratynska, 1954
Rhapis excelsa	+	greenhouse	Maeda, 1954
Roystonea elata	+	field	Barada & Halos, 1980
Sabal palmetto	+	field	Hood, 1964; Meador, 1977
Syagrus sp.	-	field	St. John, 1980
Washingtonia filifera	+	field	This project

Growth Response and Outplanting Experiments

Mycorrhizae have frequently been shown to improve plant growth over that of non-mycorrhizal controls. The magnitude of the mycorrhizal growth response depends heavily on such factors as the species of plant and the native fertility of the soil used in the experiment. The way in which the growth response is brought about is clearly nutritional in most cases. VAM can greatly improve uptake of phosphorus and certain micronutrients, especially zinc and copper. Growth responses in *J. bataua* were described by St. John (1988).

Of equal importance with performance in the nursery is performance after outplanting. Inoculation with VAM fungi has been shown to improve outplanting performance in other crops (Menge et al. 1978). Experiments were carried out at the Peruvian site to assess both growth response and the effect of inoculation on survival and performance at outplanting.

In evaluating the role of VAM in growth rate of palms, we worked with both forest palms and agronomic species. Our collaborators believed that these experiments, which have not been reported for any of the palms at their stations except *B. gasipaes* (Janos 1977) and *J. bataua* (St. John 1988), were of considerable importance. The Peruvian experiments combined tests of growth response and performance after outplanting. Seedlings of the five palm species were sown in the nursery in a soil previously fumigated with methyl-bromide with 2% chloropicrin applied at a rate of 600 kg/ha. Plants were placed in plastic

bags containing sterilized soil when they had three leaves. Treatments included inoculated and non-inoculated soil. Inoculum was applied at a rate of 50 g/bag and consisted of soil, fresh roots, and litter from *Inga edulis*. An infusion from soil without mycorrhizal propagules but with other soil microorganisms was applied to the non-inoculated soil. After 4 months in the nursery (11 for *J. bataua*), plants were outplanted to the field. Each species except J. bataua had 50 plants per treatment.

Euterpe oleracea: Four month old seedlings were outplanted in January 1987. Figure 1 indicates the large difference in survival rate between inoculated and uninoculated plants, as assessed in November, 1988. There were also differences in performance, which were decreasing on average as the experiment matured and as uninoculated plants died. Data on the differences in performance were not available from our Peruvian collaborator at the time of this report.

Jessenia bataua: The experiment was limited to a small number of plants because of the difficulty of obtaining seeds and of bringing a suitable group of uniform seedlings to outplanting size. Genetic diversity in forest-collected seeds and the slow rate of germination and growth have complicated all the experiments with J. bataua. The seedlings, already eleven months old, were outplanted in August 1987. Out of nine inoculated and nine uninoculated plants that were outplanted to the field plot, eight

inoculated and six uninoculated plants survived at harvest in late 1988. Figure 2 shows the results. The Wilcoxen rank sum test, applied to height at the longest leaf, showed inoculated plants to have performed better than uninoculated ($p<0.05$). Note that a comparison of this type considers only the plants that survived, making the difference in performance appear smaller than it really is.

Bactris gasipaes: Four month old seedlings were outplanted in January 1987. This experiment was still in progress in November 1988. Although the inoculated plants were considerably larger at that time, the uninoculated plants were closing the gap, presumably because they had become mycorrhizal from native soil inoculum and spread of the fungus from inoculated plants.

Orbignya martiana: Four month old seedlings were outplanted in October 1987. No clear differences developed in between inoculated and uninoculated *O. martiana* by November 1988, although visually the inoculated plants appeared somewhat more robust. No plants of either treatment died, but some inoculated plants were stolen. No significant difference existed in number of leaves, number of compound leaves, or length of the longest leaf. All work to date with *O. martiana* has indicated a minimal or nonexistent mycorrhizal response.

Factorial Growth Response Experiment

One of our first priorities in the Brazilian work was the establishment of a factorial experiment examining mycorrhizal growth response of *Jessenia bataua*, *Orbignya martiana*, and *Euterpe oleracea* inoculated with the mycorrhizal fungus *Gigaspora Margarita*. The experiment, detailed in a CEPLAC internal report, was to be carried out at a range of fertility levels. The experiment was designed and established during a site visit by St. John in 1986. Shortly thereafter two of the key personnel left CEPLAC and the experiment failed for lack of attention. Among the problems was improper watering by unsupervised station personnel, resulting in attack of the seedlings by a parasitic fungus. The remaining Brazilian collaborators made the decision to abandon the experiment when the number of surviving plants became too low in some treatments.

Less elaborate growth response experiments were conducted or attempted in the United States. The experiment with *J. bataua* (St. John 1988), although carried out before the formal initiation of this funding, was designed as a part of the project and has been reported as such. Subsequent work with *J. bataua* and *Oenocarpus bacaba* suffered from failure of facilities that had to be improvised after St. John's change of institution. An experiment with *J. bataua* and *O. martiana* between August 1987 and February 1988 was severely damaged by a wind that tore the roof off of the greenhouse. Too few *J. bataua* remained for statistical analysis, but enough *O. martiana* survived to allow

examination of the results. There was no significant effect of mycorrhizal inoculation on number of surviving plants or height at the longest leaf. The exposure to unfavorable temperatures during the windstorm preclude publication of this experiment, but it is interesting that the result supports the Peruvian experiments.

NURSERY PROCEDURES FOR ESTABLISHMENT AND MAINTENANCE OF THE MYCORRHIZAL ASSOCIATION

The highest priority with regard to developing nursery techniques has been discovering the reason for the failure of mycorrhizal infection in the nursery. Our hypothesized reasons for a lack of infection included:

Lack of native VAM inoculum in the nursery potting soil, which could be traced to either a lack of inoculum at the source or a loss of inoculum in storage or handling;

Inhibition of root colonization in the nursery as a result of heavy fertilization, use of pesticides, or high root temperature in unshaded conditions.

Fertilization and pesticides, undoubtedly of importance in temperate-zone nurseries, were considered of secondary importa after initial interviews of the nursery managers indicated tha little or no fertilization or pesticide use is carried out with palms at their nurseries.

LACK OF INOCULUM AT THE SOURCE OF NURSERY POTTING SOIL; LOSS OF INOCULUM FROM STORAGE OR HANDLING OF NURSERY POTTING SOIL

These factors were all investigated in the same experiment. A test for viable inoculum, using corn as a bioassay plant, was carried out on nursery potting soil collected in the manner routinely used by the palm nursery at Una, Bahia, Brazil. Our expectation was that stored soil would show less colonization than unstored if storage resulted in a loss of inoculum, and that sieved soil would show less colonization than unsieved if sieving resulted in a loss of inoculum. Finally, if the field soil were poor in inoculum no treatment would show heavy colonization. The soil was separated into four portions to represent the hypotheses under investigation. Half of the collection was planted immediately and half was stored for thirty days. Those portions were each divided into halves that were sieved or not through a 10 mm screen normally used by the nursery. The soil was collected on July 28, 1986, and the unstored treatments set up on July 29, 1986.

The results are shown in figure 3. The statistical analysis of root colonization is made rather complex by the non-normal distribution of the data. The analyses were carried out with log-transformed data in the corn bioassay, or with non-parametric statistics (St. John and Koske 1988) in other experiments except as indicated.

All root colonization values were low, indicating a serious lack of native inoculum in the pasture soil used by the nursery. This is not surprising; while the soil came from a range of depths, most of it came from below 30 cm. Mycorrhizal colonization is know to be concentrated near the surface (St. John and Machado 1980, Jakobsen and Neilsen 1983) and any surface soil would have been greatly diluted by deeper soil. Further, the soil was collected at a cut bank that is exposed to repeated wetting and drying cycles and to direct sunlight, all of which could be expected to adversely affect any inoculum near the vertical surface of the bank.

The most obvious treatment difference was between stored and unstored soil. Various investigators in temperate climates have found that storage of topsoil results in a loss of VAM inoculum (Miller et al. 1985, Warner 1983). While this decrease in viability was rapid in comparison to most temperate studies, it is not unusual for biological processes, such as the ones associated with the loss of viability, to occur rapidly in the tropics.

Sieving may have reduced inoculum potential in the unstored samples as implied by the significant interaction. However, the effect was reversed in the case of stored samples and it would be unwise to attach importance to sieving without follow-up experiments.

INHIBITION OF ROOT COLONIZATION DUE TO HIGH ROOT TEMPERATURE IN UNSHADED CONDITIONS

The operators of the nursery at Una, Bahia, rear almost all palm seedlings under shade. At Yurimaguas and Manaus, where St. John has made past observations, palm seedlings are routinely grown in full sun. The plants are often grown in perforated black plastic sacks, and the root zone temperature could easily reach temperatures not found in the root zone of wild-grown plants. VAM fungi are commonly sensitive to temperature; 50°C was sufficient to kill spores of all species tested by Menge et al (1979) and temperatures above 35°C were inhibitory to all but one species tested by Schenck and Smith (1982). Temperatures above 29.5°C were inhibitory in Oregon (Parke et al. 1983).

Experiments carried out at Yurimaguas examined the effects of direct sunlight (conventional nursery methodology) vs. shaded containers (with tops exposed to full sunlight; see color photo 3). Experiments were established with *E. oleracea*, *O. martiana*, *J. bataua*, *B. gasipaes*, and *Oenocarpus sp.* collected in native forest near Yurimaguas. There were 15 plants per treatment except that for *E. oleracea* there were 50 per treatment. All *Oenocarpus* seedlings died for lack of water during a general strike. Inoculated plants were established as in the previous experiment. When plants had three leaves they were placed in plastic bags containing inoculated soil and the bags were placed in the field.

Of 50 seedlings of *E. oleracea* per treatments, 29 inoculated and 7 non-inoculated plants survived. In the shaded treatment, average soil temperature was 31.5°C while in the treatment without shade the soil temperature was 36.4°C. Five months after transplanting, treatment related differences in plant height and number of leaves were evident (Tables 5 and 6). Plants were harvested and root length, dry weight of shoots and roots, and mycorrhizal infection were determined. Results for *O. martiana* and *B. gasipaes* are shown in tables 7-12; see also Figure 4.

TABLE 5. EFFECTS OF ROOT SHADING ON GROWTH OF *EUTERPE OLERACEA*

	# LVS	HT CM	SHOOT D W	ROOT D W
			SHADED	
MEAN	4.7	27.3	3.2	1.29
SAMPLE SIZE	15.0		15.0	
ST. ERR.	0.2		0.3	0.1
Wilcoxen rank sum test, p	<0.01		<0.01	
	# LVS	HT CM	SHOOT D W	ROOT D W
			UNSHADED	
MEAN	2.2	15.7	1.3	0.29
SAMPLE SIZE	13.0		13.0	
ST. ERR.	0.1		0.1	

TABLE 6. EFFECTS OF ROOT SHADING ON BELOW GROUND PROPERTIES OF *EUTERPE OLERACEA*

	ROOT LN, CM	SHADED % COLON.	COLON. RT LENGTH
MEAN	42.0	34	14
SAMPLE SIZE	15.0		
ST. ERR.	1.9		
Wilcoxen rank sum test, p	<0.01		

	ROOT LN, CM	UNSHADED % COLON.	COLON. RT LENGTH
MEAN	20.4	7	1.4
SAMPLE SIZE	13.0		
ST. ERR.	2.0		
Wilcoxen rank sum test, p.	<0.01		

Note: Statistical parameters not calculated where raw data were unavailable from collaborator at the time of writing.

TABLE 7. EFFECTS OF ROOT SHADING ON GROWTH OF *BACTRIS GASIPAES*

	# LVS	HT CM	SHOOT D W	ROOT D W
			SHADED	
MEAN	5.9	27.6	8.08	8.52
SAMPLE SIZE	10			
ST. ERR.	0.5	1.7	1.2	1.3
Wilcoxen rank sum test, p	0.01	NS	NS	<0.05

TABLE 8. EFFECTS OF ROOT SHADING ON BELOW GROUND PROPERTIES OF *BACTRIS GASIPAES*

	RT LN, CM	ACUAL. SPORES	% COLON	COLON CM
			SHADED	
MEAN	40.4	21.1	22.3	9.2
SAMPLE SIZE	10			
ST. ERR.	2.8	4.3	8.6	3.3
Wilcoxen rank sum test, p	NS	<0.01	<0.01	<0.01

TABLE 9. EFFECTS OF ROOT SHADING ON GROWTH OF *BACTRIS GASIPAES*

	# LVS	HT CM	SHOOT D W	ROOT D W
			UNSHADED	
MEAN	2.20	24.90	5.65	4.67
SAMPLE SIZE	10			
ST. ERR.	0.49	1.79	0.77	0.79

TABLE 10. EFFECTS OF ROOT SHADING ON BELOW GROUND PROPERTIES OF *BACTRIS GASIPAES*

	RT LN, CM	ACUAL. SPORES	% COLON	COLON CM
			UNSHADED	
MEAN	24.90	0.6	6.5	2.1
SAMPLE SIZE	10	10	10	10
ST. ERR.	1.79	0.17	4.3	1.1

TABLE 11. EFFECTS OF ROOT SHADING ON GROWTH OF *ORBIGNYA MARITIANA*

	# LVS	PLANT HT, CM	SHOOT D W	ROOT D W
			SHADED	
MEAN	6	58.3	45.07	1.59
SAMPLE SIZE	8			
ST. ERR.	0.81	9.0	10.51	0.40
Wilcoxen rank sum test, p	NS	NS	NS	NS
	# LVS	PLANT HT, CM	SHOOT D W	ROOT D W
			UNSHADED	
MEAN	7.17	49.08	41.27	2.23
SAMPLE SIZE	6	6	6.00	6.00
ST. ERR	0.66	5.20	10.87	0.31

TABLE 12. EFFECTS OF ROOT SHADING ON BELOW GROUND PROPERTIES OF *ORBIGNYA MARITIANA*

	ROOT LN, CM	% COLON	COLON CM
		SHADED	
MEAN	710	6.2	48.3
SAMPLE SIZE	8		
ST. ERR.	187	2.5	20.5
Wilcoxen rang sum test, p	NS	NS	NS
	LN, CM	ROOT COLON	% COLON CM
		UNSHADED	
MEAN	1085	6.3	52.8
SAMPLE SIZE	6		
ST. ERR.	217	2.2	21.3

The *O. martiana* used in the experiment were from an important collection from Minas Gerais, Brazil. They are to be thinned to 2 x 2 meter spacing and kept in place as part of the germplasm bank.

In addition to determining the reasons for a lack of colonization, we consider it important to find and produce cultures of VAM fungi able to produce good growth responses in the conditions of nursery and cultivation that we expect to encounter. This appeared as secondary objectives and was considered of high priority by all of our collaborators. Spore collection and screening projects have been conducted in Brazil as joint efforts with other funding, and are beginning in Peru. Another related project in Peru was the selection of effective field inoculum. A bioassay of several field-collected materials compared litter, roots, and soil from Inga edulis and roots from

a degraded pasture. The pasture roots proved superior in initiating new root colonization.

PUBLICATIONS ON US AID-FUNDED RESEARCH

The results of the US AID-funded research on forest palms as tropical resources are to be published as a series of papers and reports. These will be sent to US AID as they appear.

WORK IN PERU

The effort in Peru has been a resounding success, due in large part to the enthusiasm and resourcefulness of our Peruvian collaborator, Mr. Pedro Ruiz of the International Potato Center. The Yurimaguas group has a long-standing interest in mycorrhizae. The initiation of this project played an important role in translating that interest into a series of nursery and field projects, which by the time of this writing have expanded well beyond the scope of the forest palm work. In addition to Mr. Ruiz, at least two graduate students from North Carolina State University now have mycorrhizal components to their projects. Perhaps more importantly, Mr. Ruiz has become a recognized educational resource in eastern Peru since attending our training session in Brazil. He has repeatedly been sought out by Peruvian students wishing to work with mycorrhizae, and during St. John's 1988 visit two students from Peruvian universities were at the station for training from him. In addition, several station

personnel and students were benefitting from his expertise. The initial results of the shading experiments were so successful that the experimental treatment was adopted as standard nursery practice before the first experiment was even concluded.

Our training of Mr. Pedro Ruiz took place at CEPLAC in Brazil in July of 1986. St. John designed and carried out most of the training. Rogerio Dias, then of CEPLAC, provided cultures of spores and examples of mycorrhizal plant material in addition to arranging necessary laboratory and microscope facilities at CEPLAC. An outline of the course is given in an appendix. The success of the training program is evident from the substantial research output of Mr. Ruiz since 1986, and from the fact that he has made Yurimaguas into a sort of training center for other young Peruvians interested in working with mycorrhizae.

The agreement with the Peruvian collaborators reflected not only the objectives of our original proposal, but their own priorities and their wish to generalize the mycorrhizal work beyond forest palms. The objectives under our agreement are essentially those given in the general treatment above, with the addition of some cultivated palm species in some of the experiments.

Experimental work at Yurimaguas has concentrated on survival and performance after outplanting and on the effects of temperature on mycorrhizal colonization of roots. Initial results of some experiments have been reported above. Some of

the material and data await analysis in Yurimaguas and California.

WORK IN BRAZIL

The effort in Brazil has been less successful than that in Peru. This is due primarily to the loss of two key personnel and the transfer of a third key person to a position that distanced him from the project. The project was thus essentially left without representation at CEPLAC. The most serious loss was the complex factorial experiment on effects of inoculation at several fertility levels. This experiment was designed by St. John, Dias, Ruiz, and CEPLAC personnel during St. John's 1986 site visit. Logistic arrangements and initial preparation of the soil and shadehouse space were made at that time. other matters such as planting and installation of initial treatments had to await seed germination and the availability of personnel and materials, and were left in the care of Dias. Unfortunately Mr. Dias left CEPLAC soon thereafter, as did Dr. Percy Caballa. Dr. Ronald Alvim, who had an early administrative role in the project, was transferred within CEPLAC and could no longer participate. Erratic communications and a lack of substitute personnel at CEPLAC prevented the reestablishment of the experiment, and it had to be abandoned soon after Dias's departure.

Fortunately, the less complex but equally important experiment on inoculum in nursery soil was successfully completed, as were some of the ancillary and joint subprojects.

The germplasm bank at Una faltered when Dr. Ronald Alvim, who was to be in charge of it, transferred within CEPLAC. Some early seed supplied by the joint NYBG-CENARGEN effort was used in an agroforestry experiment at Una. An important accession of an *Orbignya* species is still in temporary quarters in a shade house at the station, and was located with great difficulty during St. John's November 1988 visit.

Dr. Paulo Alvim has located an established Brazilian mycorrhizal researcher at the Federal University of Bahia in Salvador, Mr. Osvaldo Manoel Santos. Mr. Santos has worked with St. John in the past and plans a reconstructed fertility experiments. A joint communication from Santos and St. John to Dr. Alvim detailed the recommended modifications, slightly shifting the focus of the experiment and helping to prevent a repetition of the earlier misfortunes.

As was true in Peru, our Brazilian collaborators wished to include in the experiments palm species of proven economic value. Thus our inclusion of *Euterpe oleracea* and *Bactris gasipaes* in the factorial experiment. The agreement with CEPLAC reflects the priorities of the original proposal and of the Brazilian collaborators.

WORK IN THE USA

Certain growth response experiments were carried out in the United States, both in anticipation of the project (St. John 1988) and after its initiation in South America. An

administrative matter resulted in some unanticipated practical difficulties. St. John changed institutions between the submission of the proposal and its funding. Informal agreements with the new institution, the Laboratory of Biomedical and Environmental Sciences at UCLA, had indicated that the mycorrhizal portion of the project could easily be transferred there. Only after the project was approved and the move completed did a surprising laboratory policy become clear: no work could be carried out there unless it was based in an arid climate. Thus the U. S. portion of the project was somewhat orphaned, with mycorrhizal work being carried out on a consultant basis in rather makeshift facilities. While the facilities performed well over most of their term of usage, a single failure of a heating system in 1985 and a single damaging wind storm in 1987 each did sufficient damage to these delicate tropical plants to effectively destroy many months of work in each case.

The objectives being pursued in California were supplementary investigations of possible growth responses of inoculated and uninoculated palm seedlings. A commercially-available inoculum (*Glomus intraradices*) was used in each instance, and the soil was similar to that used by St. John (1988). Experiments were initiated at various times with *J. bataua*, *O. maritima*, *E. oleracea*, and *B. gasipaes*. All but *B. gasipaes*, and to some extent, *O. maritima* proved extremely sensitive to the low temperatures experienced during the incidents described above,

and were killed outright or set back so severely as to be of little experimental value.

The results of one portion of one of these experiments may be of use. In that experiment there was no growth response to inoculation. Low temperature, physiological shock, and numerous other factors could have introduced this result regardless of an advantage in phosphorus uptake that the symbiosis may have been providing, and the advisability of accepting the results remains dubious. It is interesting, however, that Ruiz reached the same conclusion with this species at Yurimaguas.

The most valuable information provided by the experiences in California relate to the sensitivity of these plants to handling and to environmental factors. The overwhelming factor controlling growth of the plants was not any experimental variable, but recovery of the plants from transplanting. Half or more of all plants simply failed to grow further after transplanting. Most eventually died, some later began to grow, but in no case were the plants uniform enough in behavior to realistically consider them in the same experiment. Because of these problems, nurserymen in California who work with tropical palms simply arrange their operations to avoid transplanting. The tropical nurseries also plant directly into the only nursery container. Mycorrhizal experiments could be devised to accommodate these restrictions, but the ones carried out here were conceived with a transplant stage. It is possible, even likely in view of the Peruvian results, that pre-inoculation with

VAM fungi could reduce this transplant shock. However, that would not directly test the objectives of this project and no follow-up on that point has been planned.

CONCLUSIONS

J. bataua is extremely variable from currently available sources. It is very slow growing and thus holds little promise as a primary crop. However, it may be suitable for use as an understory crop in agroforestry, where its ability to grow in low light intensity and very poor soil will be valuable.

In vitro propagation appears possible with *Orbignya*, but cryopreservation is somewhat more tenuous. The specialized and expensive process of sorting out these methods requires more attention than current project personnel can give it. We recommend it as a high priority for the future, when specialists become available in host countries.

Most if not all palm species are capable of supporting the vesicular-arbuscular type of mycorrhizal symbiosis. Clear improvements in growth and survival were brought about by nursery inoculation of *Euterpe oleracea*, *Jessenia bataua*, and *Bactris gasipaes*. No clear response was evident in *Orbignya martiana*.

Mycorrhizal inoculum was of poor quality in the soil used by the Brazilian nursery, and was further degraded by storage of the soil. Lack of inoculum is most probably the primary cause of mycorrhizal failure in these nurseries.

Exposure of containers to direct sunlight is a normal practice at some tropical nurseries, but is detrimental to plant growth and mycorrhizal colonization. Substantial improvements were brought about by shading of pots without shading shoots.

Differences were evident in *Euterpe oleracea* and *Bactris gasipaes*, but not in *Orbignya maritima*, which generally failed to respond to VAM inoculation. Temperature effects appear to be an important cause of mycorrhizal failure in these nurseries.

Collection and identification of effective fungal isolates is an important future priority.

Enthusiasm of our collaborator, plus a general informality and *esprit de corps* at the Yurimaguas station made our project there a resounding success, despite a lack of resources and lack of experience with mycorrhizae at the station. Conversely, administrative difficulties, rather than lack of facilities at CEPIAC made the results there somewhat disappointing.

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Figure 1

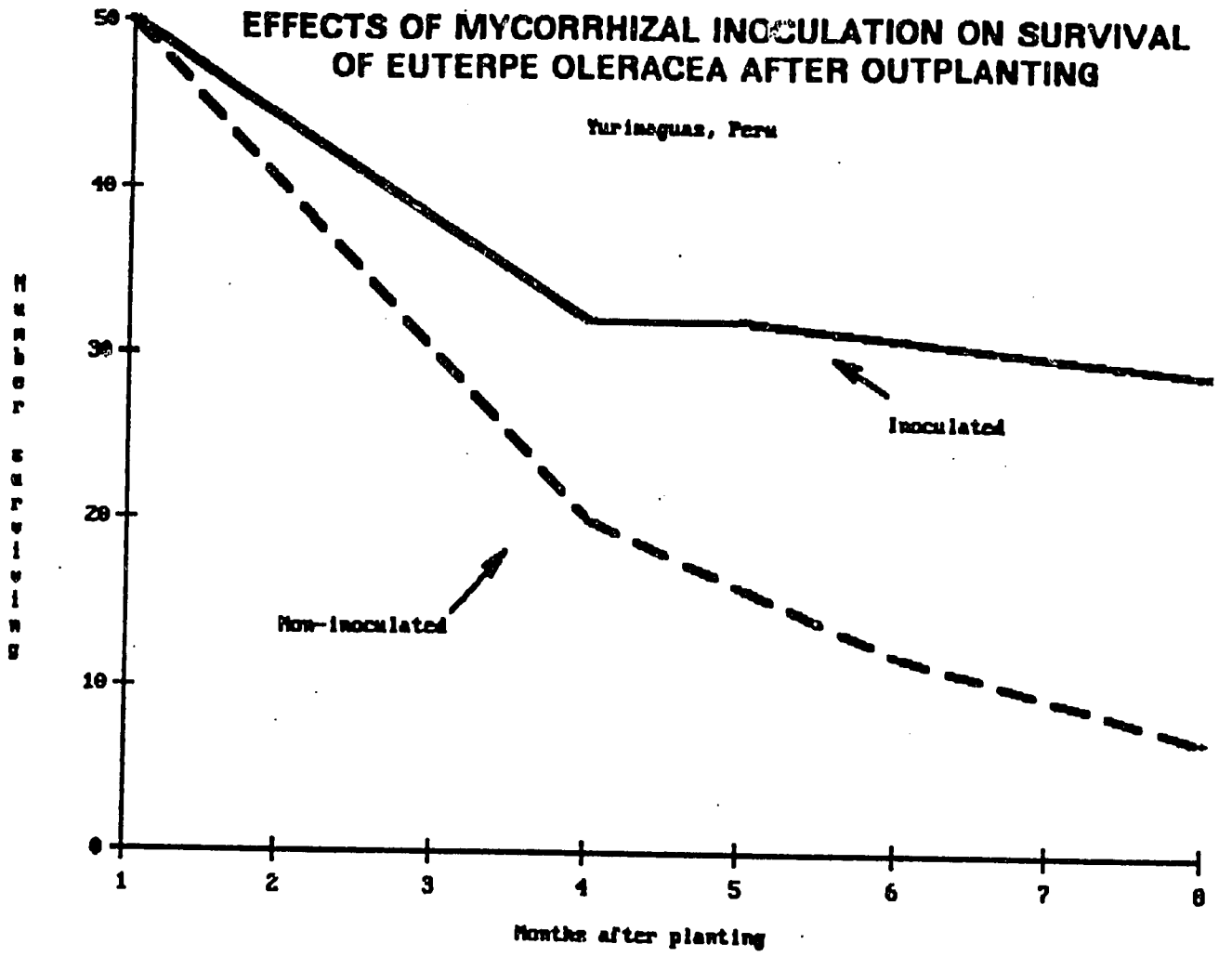
EFFECTS OF MYCORRHIZAL INOCULATION ON SURVIVAL
OF EUTERPE OLERACEA AFTER OUTPLANTING
Yurimaguas, Peru
Number surviving
50
40
30
20
10
0
1
2
3
4
5
6
7
8
Months after planting
Inoculated
Non-inoculated

Figure 2

EFFECTS OF MYCORRHIZAL INOCULATION ON GROWTH OF JESSENIA BATAUA AFTER OUTPLANTING

Yurimaguas, Peru

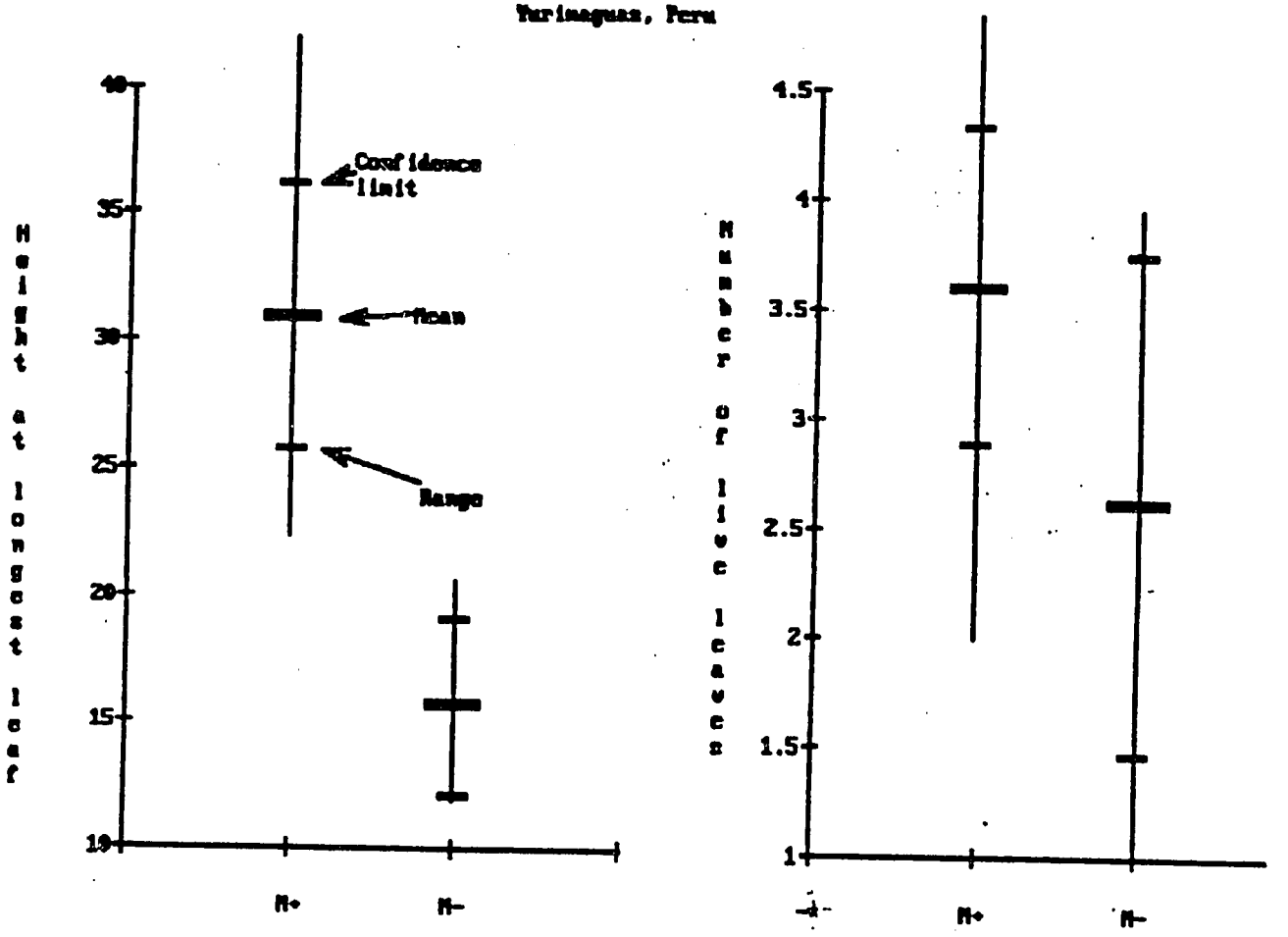

Figure 3

BIOASSAY OF MYCORRHIZAL INOCULUM IN PALM NURSERY SOIL AFTER FOUR TREATMENTS

Una, Bahia, Brazil

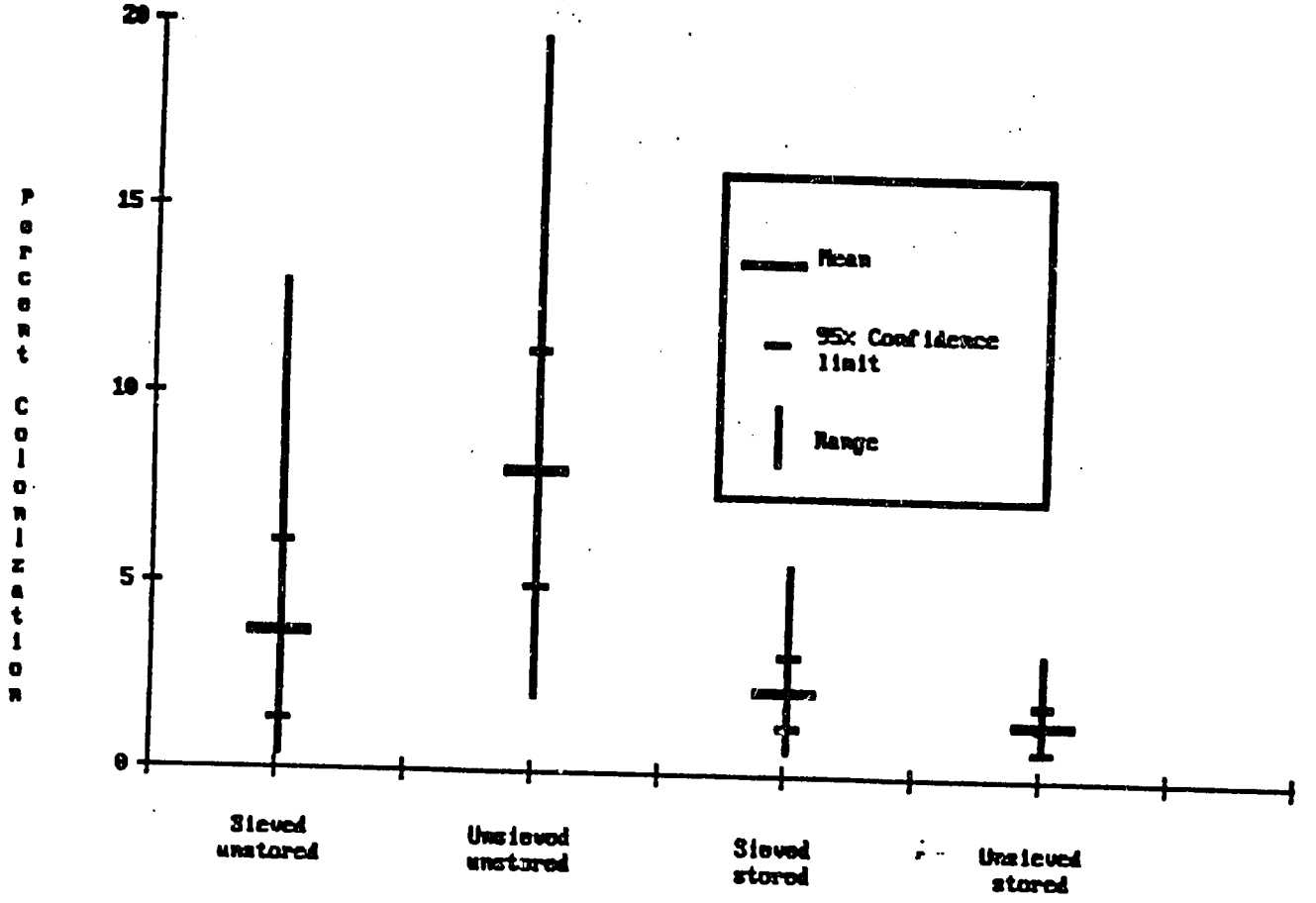

Figure 4

EFFECTS OF SHADE ON GROWTH AND MYCORRHIZAL DEVELOPMENT OF BACTRIS GASIPAES

Yurimaguas, Peru

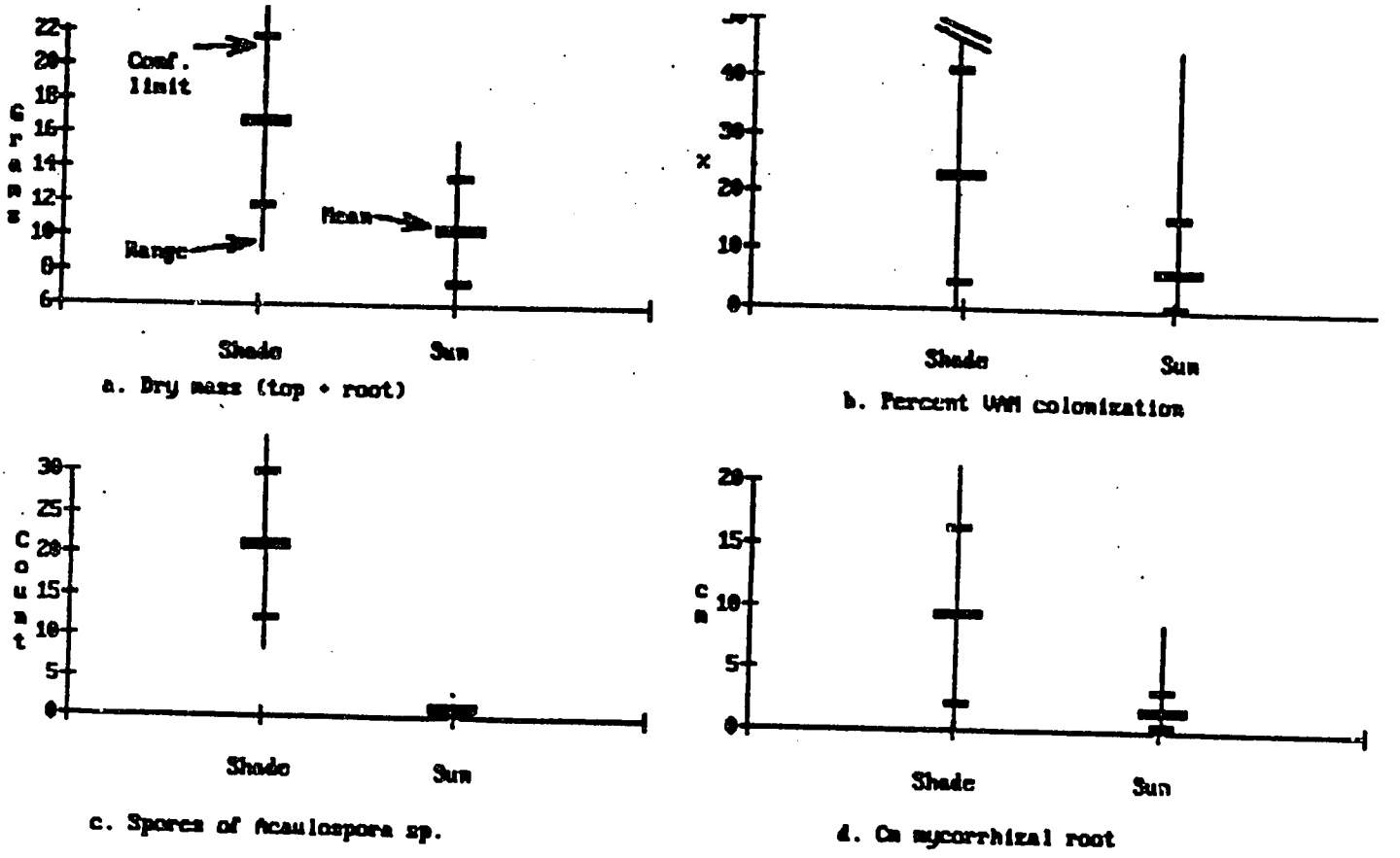

Photo 1. Variability of J. bataua in an agroforestry plantation, Una, Bahia, Brazil.

Photo 2. A Minas Gerais collection of O. martiana with collaborator Roberto Euclides Lima y Borges. Una, Bahia, Brazil.

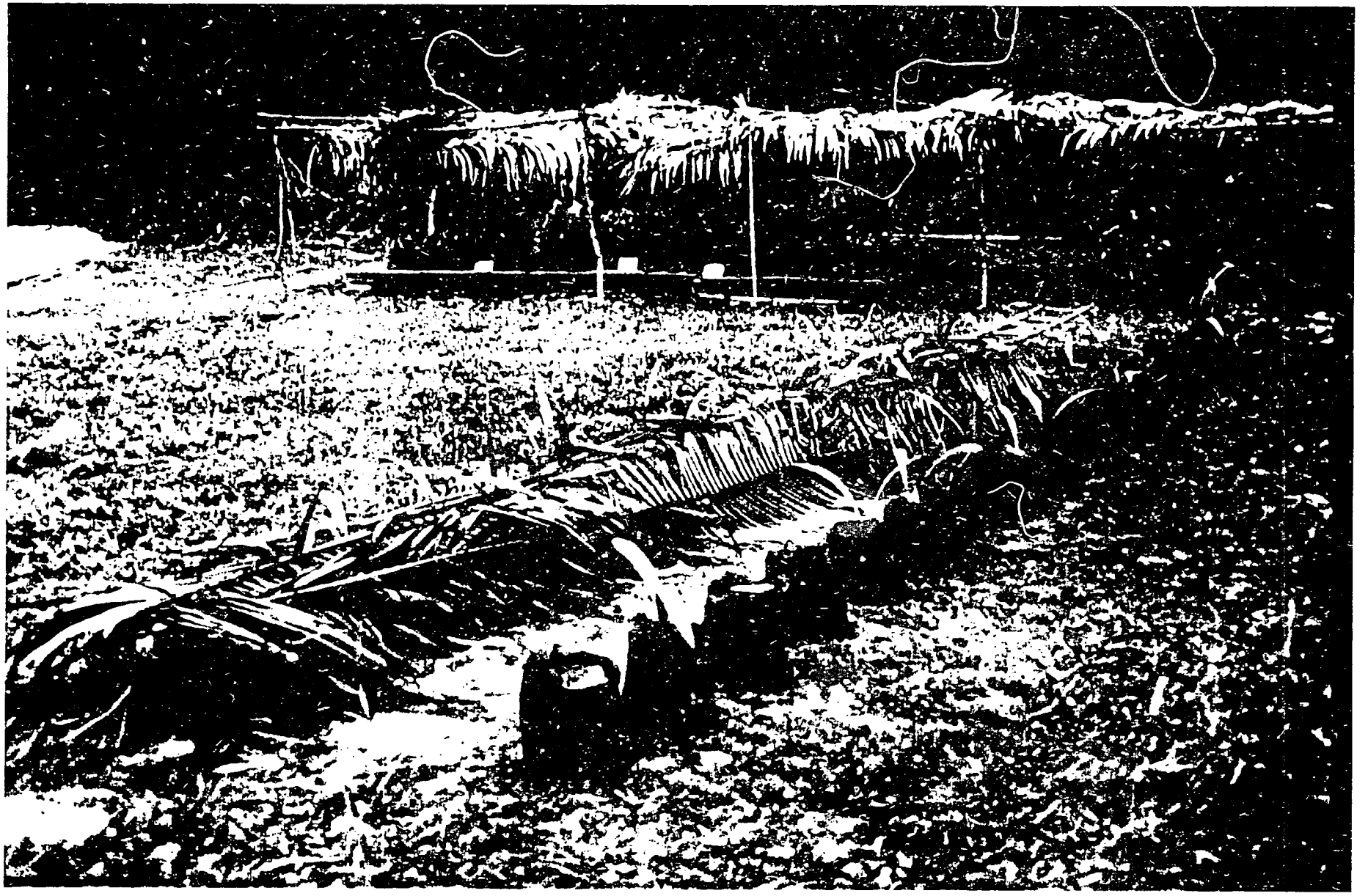

Photo 3. Root-shading experiment with E. oleracea, Yurimaguas, Peru.

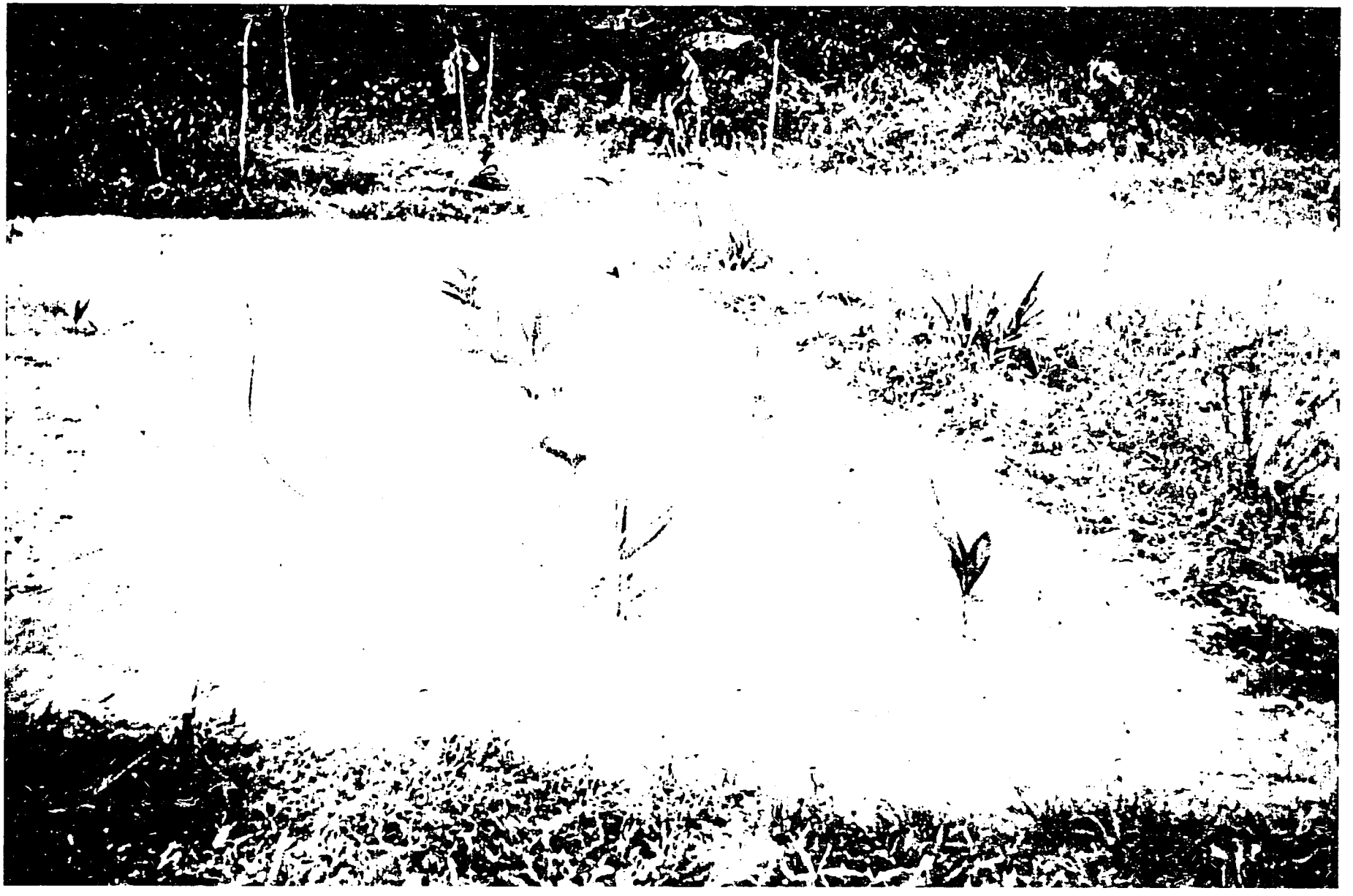

Photo 4. Outplanting performance of J. bataua, Yurimaguas, Peru. Inoculated plants are on the left.

Photo 5. Outplanting performance of B. gasipaes, Yurimaguas, Peru. Inoculated plants are in the back rows.

Photo 6. Peruvian collaborator Pedro Ruiz and a visiting student from Lima examine her mycorrhizal experment with Bixa. Yurimaguas has become the focus of mycorrhizal research beyond the scope of this project since Mr. Ruiz was trained in 1986.

Photo 7. Growth of *Jessenia bataua* with mycorrhizal innoculation (left) and without (right).

APPENDIX I

Outline of mycorrhiza training program for Peruvian collaborator.

A. Review of classic literature

1. Butler 1939, Mosse 1959, Nicolson 1959, 1960, Gerdemann and Nicolson 1963, Nicolson 1967, Gerdemann.

B. Other resources

1. Methods books
   a) Schenck
   b) Powell

2. Directory of mycorrhiza researchers

   a) Hacskaylo and Tompkins 1975
   b) Saif
   c) CAB abstracts
   d) other

C. Preparing roots (50% time)

1. Clearing and staining

   a) Field collecting
   b) Clipping roots
   c) Vessels for clearing/staining
   d) Removal of cytoplasm (KOH)
   e) Bleaching
   f) Acidification
   g) Staining
   h) Destaining

Identification of VAM confusing structures

2. a) Hyphae
   b) Arbuscules
   c) Vesicles
   d) Chitrids
   e) Rhizoctonia

D. Spores (20% time)

1. Field collection
2. Handling samples
3. Extraction of spores
   a) Wet sieving and decanting
   b) Ellutriator
4. Centrifugation
   a) Sucrose gradient

5. Quantification
6. Identification
   a) To genus
   b) Preparing specimens for a taxonomist

E. Setting up pot cultures (20% time)

1. Growing conditions
2. Soil
3. Source of spores
4. Methods of inoculation
5. Fertilization
   (1) Appropriate nutrient solutions

F. Setting up experiments (10% time)

1. Controls
   a) High P controls
   b) "Washings"

2. Choice of conditions
   a) Plant growth conditions
   b) Fertilization
      (1) Appropriate nutrient solutions
   C) Soil

G. Supplies required

1. Methods book

2. Root preparation
   a) Safety equipment, electric hotplate, pan wash bottles, chemicals, KOH, lactophenol, liquid phenol, lactic acid, trypan blue, ammonium hydroxide, glycerin, hydrogen perioxide, HCl

3. Micros supplies
   a) Scopes
      (1) Dissecting scope
      (2) Compound scope
   b) Materials
      (1) Petri dishes, forceps, needles, pi -pumps,disp. pipettes, counting dish
   c) Quantification
      (1) Spores
      (2) Colonization
         (a) Grids

4. Sieving spores
   a) Sieves
      (1) About 45 microns, around 110, around 250-300, around 1000, around 2 mm
   b) Decanting
      (1) Lrge graduates, funnels, misc containers

c) Centrifuging
   (1) Centrifuge with large vessels, 50% sucrose soln-pepare ahead of time, pipettes, sieve for washing spores

5. Pot culture
   a) Materials
      (1) Containers, bricks, basin, watering wicks, funnels, soil
   b) Fertilization

6. Experiments

   a) Soil
   b) Containers
   c) Fertilizers
   d) Greenhouse/growth chamber
   e) Controls
      (1) Filtering: "washings"
   f) Randomization
   g) Statistical analysis

APPENDIX II

SELECTED PUBLICATIONS RESULTING FROM THIS PROJECT

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Biodiversity and Conservation **1**, 112–119 (1992)

Experimental investigations into the feasibility of *ex situ* preservation of palm seeds; an alternative strategy for biological conservation of this economically important plant family

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Given the widespread belief that the conservation of palms, especially the large-trunked species, is only accomplished through *in situ* preservation or in plantations, this paper explores the feasibility of a third approach, i.e. cryogenic preservation of their seeds *ex situ*. Seeds of the following palm species were subjected to air-drying to assess their tolerance of dessication: *Washingtonia filifera* (L. Linden) H. Wendl.; *Sabal mexicana* Mart. (syn. S. texana; Zong, 1990); *Schippia concolor* Burret; *Orbignya cohune* (Mart.) Dahlgren ex Standley; *Acoelorraphe wrightii* (Griseb. & H. Wendl.) H. Wendl. ex Becc.; *Desmoncus orthacanthos* Mart.; *Attalea crassispatha* (Mart.) Burret; *Zombia antillarum* (Desc.) L.H. Bailey; *Pinanga malaiana* (Mart.) Scheff.; *Pinanga aff. polymorpha* Becc.; *Daemonorops verticillaris* (Griff.) Mart. Of these, only two (*W. filifera* and *S. mexicana*) survived drying to moisture contents around 5% (fresh weight basis). Seeds of the remaining spp. would be difficult or impossible to conserve *ex situ* in seedbanks or cryostores. Data are presented to show that the response of *O. cohune* embryos to drying is similar to other recalcitrant (dessication intolerant) seeds, while seeds of *A. wrightii* may belong to an intermediate seed storage category with limited tolerance of drying. The results are discussed in relation to the inadequacy of current knowledge as a basis for decisions on the broad scale *ex situ* conservation of palm germplasm.

Keywords: palms; seeds; preservation; desiccation; survival

Introduction

Palms are amongst the most economically important plant families throughout their extensive distribution in the tropics and subtropics. As well as the familiar examples such as coconut and oil palm, currently important in commerce, there are hundreds of other palm species of economic utility (Balick and Beck, 1990). The conservation and effective utilization of palm germplasm is beset by the same problems facing other tropical families. *In vivo* collections, both in the wild and in repositories, are subject to diseases, bud mutations, human encroachment and poor management, as well as being expensive in terms of management inputs and space. The application of *in vitro* methods of *ex situ* conservation has several associated technical problems, including the relatively frequent

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need for subculture and the fact that a single protocol is unlikely to be useful to diverse genotypes (Towill and Roos, 1989).

The storage of seeds at low moisture contents (air-dry) and at low (sub-zero temperatures offers a convenient and cost-effective means of conserving plant genetic resources in the medium and long term, and is the basis of the operation of a number of seed banks worldwide, in which useful seed storage lives are expected to be of the order of at least many tens and probably many hundreds of years (Roberts, 1989). Success of this technology depends upon the ability of seeds to withstand drying to quite low moisture contents (< 10% on a fresh weight basis). Low seed moisture content not only removes the possibility of damage due to freezing injury in subsequent deep-freeze (−20° C) or cryogenic (e.g. liquid nitrogen, −196° C) storage, but also itself promotes, along with reduced temperature, increased longevity in orthodox seeds (Roberts, 1973).

Another group of plants, including several important tropical plantation species, bear seeds classified as recalcitrant (Roberts, 1973). The main characteristics of such seeds is their inability to withstand any degree of desiccation and so they cannot be kept at sub-zero temperatures. Their lifespans are relatively short and cannot be extended by the methods presently employed to preserve germplasm in seedbanks or cryogenic stores.

It seems that many palm species are propagated primarily by seed (Broschat and Donselman, 1988); and, although Corner (1966, p 183) maintains that palm seeds in general cannot withstand any degree of drying, the seeds of several species (e.g. date palm) are amenable to storage at freezing temperatures in the air-dry state (Al-Madeni and Tisserat, 1986). However, there are also examples, such as coconut, of recalcitrant seed behaviour among the Palmae (Chin and Roberts, 1980), as well as at least one instance of apparently recalcitrant seeds (oil palm) being subsequently shown to be desiccation tolerant under certain conditions (Grout *et al.*, 1983; Ellis *et al.*, 1991). This paper presents data from a study aimed at classifying seed storage behaviour in a wide range of palm species, mostly having little or no previous work reported, with a view to obtaining a reliable estimate of the proportion having seeds amenable to seed-banking or cryogenic storage.

Materials and methods

Seeds of eleven palm species were collected from sites in Central and South America and South-East Asia, as shown in Table 1, and sent to Wakehurst Place for investigation. The opportunistic nature of the collections meant that samples were often low in numbers of seeds, which were sometimes also in poor condition on arrival. Thus, the difficulties (including sporadic fruiting, obtaining export permits and the time taken to transport seeds to the laboratory) inherent in working with tropical trees precluded a rigorous experimental and quantitative analysis of all the palm seed samples received.

On arrival, fruits and seeds were stored moist in ventilated polythene bags at 16° C for up to one week before experimental treatments were begun. Where necessary, fleshy pericarps were removed by washing in tapwater. In *Orbignya cohune* the substantial hard endocarps were removed by progressive and careful application of pressure in a large engineer's vice, with further pressure splitting the seed to permit removal of the embryonic axis.

In the control lots, seeds or embryos were set to germinate without any drying, i.e. at the relatively high moisture contents at which they arrived. Seeds or embyros were dried

51

Table 1. List of palm species whose seeds were air-dried to low moisture contents, together with the geographical sources of the seed samples

Species	*Source*
Acoelorraphe wrightii (Griseb. & H. Wendl.) H. Wendl. ex Becc.	Hattieville, Belize District, Belize
Attalea crassispatha (Mart.) Burret Haiti	
Daemonorops verticillaris (Griff.) Mart.	Kepong, Selangor, W. Malaysia
Desmoncus orthacanthos Mart.	Cayo District, Belize
Orbignya cohune (Mart.) Dahlgren ex Standley	Cayo District, Belize
Pinanga malaina (Mart.) Scheff.	Pahang, West Malaysia
Pinanga aff. polymorpha Becc.	Pahang, West Malaysia
Sabal mexicana Mart. Becc.	San Antonio, Texas, USA
Schippia concolor Burret	Cayo District, Belize
Washingtonia filifera (L. Linden) H. Wendl.	San Antonio, Texas, USA
Zombia antillarum (Desc.) L.H. Bailey	Fairchild Tropical Garden, Florida, USA

to low moisture content in a room at 15% relative humidity and 15° C, in monolayers for periods up to four weeks. Throughout this paper moisture contents are quoted on a % fresh weight (f.wt.) basis; and they were measured gravimentrically, by weighing samples (whole seeds were quartered) before and after drying at 103 ± 2° C for about 17 h. Equilibrium relative humidities were measured using a Michel S-4020 dewpoint hygrometer (Michel Instruments Ltd, Cambridge, UK).

Germination tests consisted of incubating seeds (and embryos in the case of *O. cohune*) on 1% (w/v) distilled water agar in either 9 cm polystyrene Petri dishes or polystyrene sandwich boxes; in incubators maintained at 26° C, or fluctuating diurnally (33/19° C) with a 12 h thermoperiod, illumination on a 12 h photoperiod being provided by 'warm-white' (Sylvania) fluorescent tubes. Incubation was continued until it was obvious that no further germination would or could occur. Palm seed germination is often quite protracted (Loomis, 1958; Ellis *et al.*, 1985) and in this work incubation periods varied from five weeks (*W. filifera*) to more than one year (*A. wrightii*). The tests were monitored at regular intervals and germinated seeds (embryo extension > 2 mm) counted and removed. Whenever incipient drying out made it necessary, seeds were re-sown on fresh substrate.

In vitro techniques were also applied to embryos or seeds of *O. cohune*, *D. verticillaris* and the two *Pinanga* species. After extraction from fruits, seeds were disinfected for 2 min in 70% ethanol, followed by 50% bleach for 20 min. They were then rinsed five times in sterile distilled water and embryos were excised under aseptic conditions, and individually cultured on 1 ml of MS (Murashige and Skoog, 1962) medium containing 0.6% agar and 0.25% activated charcoal, in glass tubes sealed with polypropylene covers secured by rubber bands. For disinfection of excised embryos of *O. cohune*, a weaker (10–20%) solution of bleach was used. Cultures were incubated at 26° C in darkness. After 40 days germinating seeds or embryos were transferred to fresh medium and incubated at 29° C with a 12 h photoperiod.

For topographical tetrazolium staining of excised *O. cohune* embryos, they were imbibed on paper towel wetted with distilled water for 24 h at laboratory temperature

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(20–22° C) and then incubated in buffered 1% solution of 2,3,5-triphenyl tetrazolium chloride (Moore, 1973) for 24 h at 31° C in darkness. Embryos were scored as viable when they showed an overall even carmine red staining.

Results and discussion

Orbignya cohune

A sample of 36 kg of *O. cohune* fruits yielded about 600 seeds (usually one seed per fruit, but occasionally two and rarely three). The extraction technique using an engineer's vice gave over 90% undamaged seeds and 70–80% undamaged embryos, and was regarded as successful, bearing in mind the toughness of the mesocarp and endocarp.

About half the fruits appeared quite green, presumably having been harvested either at an earlier stage of maturity or closer to despatch than the remainder. Of the seeds from these fruits, the embryos had mean moisture contents of 69%, whereas the endosperm moisture contents were much lower (27%). Nevertheless, the equilibrium relative humidities of both tissues were found to be 97–98% at those moisture contents, indicating markedly different moisture sorption characteristics. Due to the the difficulties involved, initial germination tests were not carried out on the seeds, but tetrazolium staining of a small sample (n = 10) of excised embryos indicated high (100%) viability. The remaining fruits were already brown and the mean moisture contents of embryos and endosperms were 25% and 11% respectively (equilibrium relative humidity for both, 70%), with the fruit tissue at 12%, and vital staining of embryos indicated only 60% viability.

The majority of the fruits (of both ripeness levels) were dried to different levels by holding them in the drying room for periods varying from 1–28 days, giving a range of embryo moisture contents from 69% to 20%, with endosperms (and pericarp tissues) ranging from 27% to 5%. As the embryo moisture content barely fell over the first 7–10 days of drying, it is likely that they were being protected to an extent from desiccation, probably by the surrounding endosperm and fruit tissues; e.g. the embryos from green fruits took 28 days to reach 28% moisture content. With germination tests on whole seeds expected to be protracted and difficult, the excised embryo test (Ellis *et al.*, 1985) was used, in which the survival of healthy isolated embryos under non-sterile conditions is supposed to give a comparatively rapid indication of seed viability. In fact, all embryos rapidly succumbed to microbial infection and this method proved inadequate as a means of assessing viability over the range of moisture contents investigated. However, tetrazolium tests were run on small numbers of embryos (n = 10) at the same time. The results of these (Fig. 1) showed that viability remained unaffected (at 100%) by a reduction in moisture content from 69% to 46%. Below the latter, further reduction in moisture content led to a progressive reduction in viability, until at 20% moisture content it was only 50%.

The remaining green fruits were used in an attempt to monitor *in vitro* growth of isolated embryos (as described in Materials and methods). Of 20 non-dried (45% moisture content) embryos, 17 (85%) showed some extension after seven days incubation, with hypocotyl expansion and some greening following, but no further growth after 41 days. Embryos were dried rapidly (< 24 h) in the dry room to 6% moisture content and of 29 dried embryos, none showed any sign of growth after 142 days of *in vitro* culture. These

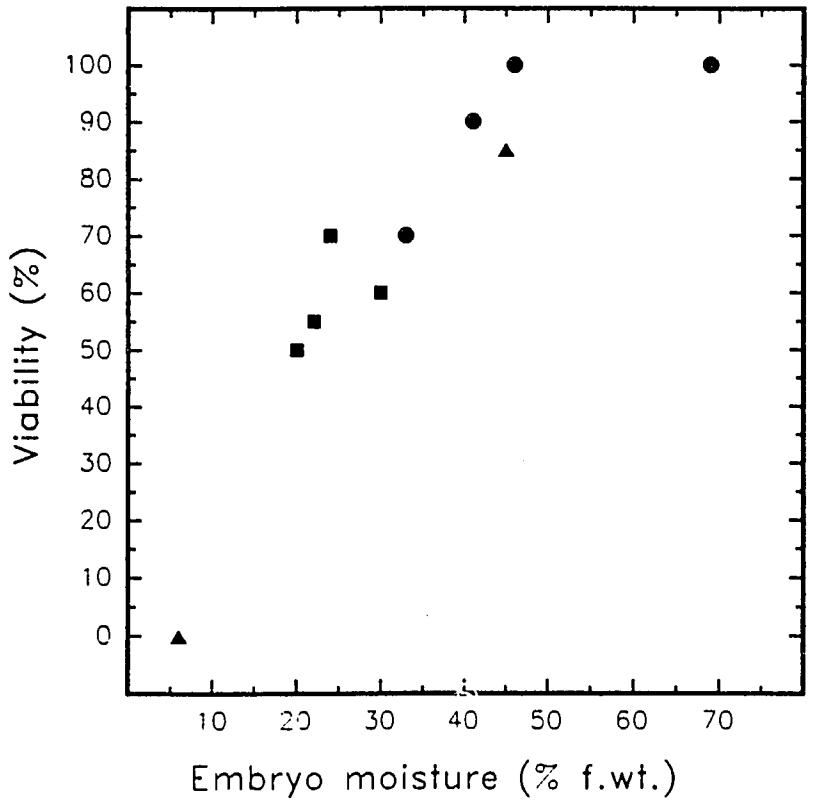

Figure 1. The effect of moisture content (% freshweight (f.wt.)) on the viability (%) of *Orbignya cohune* embryos, as measured by tetrazolium staining of embryos from green (●) or brown (■) fruits, or by *in vitro* survival and growth (▲).

results are also indicated in Fig. 1, for comparison with those obtained by drying whole fruits.

It appears that *O. cohune* axes, and therefore seeds, are not tolerant of drying to the low moisture contents required for successful cryopreservation or seedbank storage. While it is unsafe to rely on data from vital staining alone, and it would be desirable to assess survival at intermediate moisture contents quantitatively using the *in vitro* technique, the overall response to drying by *O. cohune* embryos shown in Fig. 1 is very similar to those of recalcitrant seeds (e.g. Dickie *et al.*, 1991), with reduction in moisture content below a critical value of about 45% leading to viability loss.

Sabal mexicana and Washingtonia filifera

Seed viability in the samples of both these species was high (90–95%) on arrival (with moisture contents of 12–13%), and it was not significantly reduced after drying to moisture contents as low as 4.5%. Moreover, storage at 6° C or −20° C, at any moisture content investigated, did not result in significant losses in viability, except that a small reduction (ca 10%) did occur in *S. mexicana* seeds at 12% moisture content and −20° C,

possibly due to freezing injury. The responses to drying and low temperatures of seeds of these two species were in accord with those reported for *W. filifera* and four other *Sabal* spp. by Al-Madeni and Tisserat (1986); they appear to be orthodox and should pose no problems for seedbank or cryo-storage. Indeed, it appeared from rapid ageing experiments not described here that seeds of *W. filifera* would be relatively long-lived under such conditions; as they showed no significant decline in viability over storage periods which should have removed viability completely from short-lived orthodox seeds, such as those described by Tompsett (1986).

Acoelorraphe wrightii

Whole seeds were either sown on arrival, or after drying to < 5% moisture content for 28 days in the dry room. In both cases germination was sparse, erratic and spread over 15 months. Of 100 fresh seeds, 19 germinated and a further 60 remained healthy and firm at the end of the test (presumed alive, but dormant); while 21 rotted during the test (presumed dead). Control viability may thus be regarded as 79%. Of the 90 dried seeds, only eight germinated, with a further seven presumed dormant, and 75 rotted during the test. Total viability of seeds after drying to 5% moisture content was thus 15/90, or 17%. While there was obviously some survival and germination after drying in seeds of *A. wrightii*, it was significantly reduced in comparison with non-dried seeds and the response to desiccation in this species needs further quantification, possibly employing *in vitro* techniques on excised embryonic axes to speed up viability testing. Seed banking and cryopreservation are likely to be problematical because of the losses inherent in drying seeds prior to freezing.

Attalea crassispatha

After removal from fruits, by careful use of a hacksaw, seeds germinated within two weeks of sowing. Only 14 seeds were available for investigation and of seven sown without further drying, four germinated. The remaining three rotted during the test, as did all seven dried for two weeks, and all were presumed non-viable. It seems likely that no seeds of *A. crassispatha* would survive drying to the moisture content (4.9%) reached in the drying treatment and they would not be appropriate subjects for seed banking or cryopreservation. The viability of the non-dried seeds (57%) was relatively low, and whether this was as a result of drying during shipment (moisture content on arrival, 13.7%), or some other cause is not clear.

Desmoncus orthacanthos, Schippia concolor and Zombia antillarum

Numbers of seeds received of each of these were 20, 16 and 34 respectively. Neither dried or non-dried seeds of any of them germinated, even after many months in test and all eventually rotted. It is not possible from these very limited observations to classify any one of them as recalcitrant, but it seems clear that straightforward field collection, drying and low-temperature storage is unlikely to be successful.

Daemonorops verticillaris

Cleaned whole seeds were sown *in vitro* and of 44 non-dried sown and in good condition after 14 days, 31 germinated over the next 62 days, indicating 70% viability. Of 59 seeds sown after drying to 5% moisture content, none germinated over a period of 84 days.

This result strongly suggests a lack of tolerance low moisture contents in seeds of *D. verticillaris*.

Pinanga malaiana and P. aff. polymorpha

In very small samples of seeds and excised embryos of these two species sown *in vitro*, survival and germination was only observed in freshly arrived non-dried seeds. Drying to 5% moisture content was apparently detrimental.

Concluding remarks

Of eleven species of the Palmae examined so far in this investigation, the seeds of only two (*S. mexicana* and *W. filifera*) appear to be immediately amenable to seedbank storage or cryopreservation as a means of *ex situ* conservation of genetic resources. This finding supports the commonly held view (J. Dransfield, personal communication) that considerable difficulties would attend attempts to conserve most palms *ex situ* as seeds. However, it is in contrast to data extracted from a recent literature search by Hong (1991), in which, of 21 palm species referred to, sixteen were reported as having orthodox seeds. Extrapolating, it could be estimated that only about 24% of palm species would bear seeds difficult to store (cf. 82% from the present study); but the work reported in the literature appears biased towards species from dry habitats (e.g. *Sabal* spp. and *Phoenix* spp.). Indeed, the results of the present study suggest that it is only those species regarded as belonging to dry habitats (*S. mexicana* and *W. filifera*) that bear truly orthodox seeds, easy to store at low temperatures in the air-dry state. In contrast, the remaining species examined here are characteristic of comparatively moist habitats and it appears that the seeds of none of them would be easy to preserve at low temperatures, largely due to their inability to withstand desiccation. Of the difficult seeds some would be truly recalcitrant, while others may belong to an intermediate category, in which a certain level of desiccation is tolerated, but below which loss of viability occurs. Ellis *et al.* (1991) have demonstrated this type of behaviour in seeds of at least one palm (*Elaeis guineensis* N. Jacq.). The work presented here has not allowed clear differentiation between recalcitrant and intermediate seed storage behaviour in the species examined, although the evidence available might point to the seeds of *A. wrightii* being intermediate and those of *O. cohune* being recalcitrant.

The information on seed storage behaviour generated in the present study, together with that compiled by Hong (1991) represents only 31 from a total of over 2600 palm species. As well as being very small, the sample under-represents species from moist habitats, which probably make up the great majority of palm species. Clearly, more work is needed to establish an adequate and unbiased database of palm seed storage characteristics, which could be used to assess the utility of *ex situ* seed storage in individual palm species conservation programmes. In the meantime it may be possible to suggest a rule of thumb, whereby those species of definitely dry habitats are highly likely to bear seeds that are amenable to dry, cold storage, whereas those from relatively moist habitats are likely to be difficult or impossible to store. Even the latter group will contain species with intermediate seed storage behaviour (Ellis *et al.*, 1991), which will allow medium-term preservation of viability in optimum environments. Also, the work of Chin *et al.* (1988) raises the possibility that for species whose seeds are difficult to store, there

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are nevertheless *ex situ* conservation possibilities in the cryopreservation and *in vitro* culture of their excised embryos.

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Studies on the Practicality of ex situ Preservation of Palm Seeds

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Palms are among the most economically important plant families throughout their extensive distribution in the tropics and subtropics. There are hundreds of palm species of economic utility, including the familiar examples such as coconut and African oil palm, that are currently so important in international commerce (Balick and Beck 1990).

Many of these species are little known outside their natural distributions but account for a substantial economic trade. For example, in 1979 Brazil reported over $100,000,000 of commerce derived from the harvest and sale of oil, wax, beverages, foods, fibers, charcoal, and other commodities derived from six genera of native palms: *Astrocaryum*, *Attalea*, *Copernicia*, *Euterpe*, *Mauritia*, and *Orbignya* (Balick 1985). Not included in this economic survey was the impact of palm products at the subsistence level, for example, the use of palm leaves as thatch for homebuilding. If a subsistence farmer is unable to find an appropriate thatch for his house, zinc or other similar roofing must be purchased at a substantially greater cost. Although current studies attempting to place values on these substitute products are under way in the Neotropics, there is little doubt that the total value of palm products for subsistence activities far exceeds the value of the commodities that enter the economic system. Thus, intense pressure on native palm resources, as well as habitat destruction, is resulting in a massive reduction in the genetic diversity of this plant family.

The storage of seeds at low (air dry) moisture content and at low (sub-zero) temperatures offers a convenient and cost-effective means of conserving plant genetic resources in the medium and long term. It is the basis for the operation of a number of seed banks worldwide, in which useful seed storage lives are expected to be on the order of at least many tens and probably many hundreds of years (Roberts 1989). Success of this technology depends upon the ability of seeds to withstand drying to quite low moisture contents (< 10% on a fresh weight basis). Low seed moisture content not only removes the possibility of damage due to freezing injury in subsequent deep-freeze (−20° C) or cryogenic (e.g., liquid nitrogen, −196° C) storage, but also itself promotes, along with reduced temperature, increased longevity in orthodox seeds (Roberts 1973).

The conservation and effective utilization of palm germplasm is beset by the same problems facing other tropical families. *In vivo* collections, both in the wild and in repositories, are subject to diseases, bud mutations, human encroachment, and poor management, as well as being expensive in terms of management inputs and space. The application of *in vitro* methods of *ex situ* conservation has several associated technical problems, including the

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1991), which will allow medium-term preservation of viability in optimum environments. Also, the work of Chin et al. (1988) raises the possibility that, for species whose seeds are difficult to store, there are nevertheless *ex situ* conservation possibilities in the cryopreservation and *in vitro* culture of their excised embryos.

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Table 1. Palm species examined and their suitability for ex situ *cryopreservation.*

Species	Source of Sample	Tolerance to Desiccation
Acoelorraphe wrightii (Griseb. & H. Wendl.) H. Wendl. ex Becc.	Hattieville, Belize District, Belize	somewhat
Attalea crassispatha (Mart.) Burret	Haiti	no
Daemonorops verticillaris (Griff.) Mart.	Kepong, Selangor, W. Malaysia	no
Desmoncus orthacanthos Mart.	Cayo District, Belize	no
Geonoma deversa (Poit.) Kunth	Madre de Dios, Peru	no
Jessenia bataua (Mart.) Burret	Madre de Dios, Peru	no
Orbignya cohune (Mart.) Dahlgren ex Standley	Cayo District, Belize	no
Pinanga malaiana (Mart.) Scheff.	Pahang, West Malaysia	no
Pinanga aff. *polymorpha* Becc.	Pahang, West Malaysia	no
Sabal mexicana Mart. Becc.	San Antonio, Texas, USA	yes
Salacca zalacca (Gaertn.) Voss ex Vil... rin	Village market, Malaysia	no
Schippia concolor Burret	Cayo District, Belize	no
Washingtonia filifera (L. Linden) H. Wendl.	San Antonio, Texas, USA	yes
Zombia antillarum (Desc.) L. H. Bailey	Fairchild Tropical Garden, Florida, USA	no

referred to, sixteen were reported as having orthodox seeds.

Extrapolating, it could be estimated that only about 24% of palm species would bear seeds difficult to store (cf. 86% from the present study), but the work reported in the literature appears biased towards species from dry habitats (e.g., *Sabal* spp. and *Phoenix* spp.). Indeed, the results of the present study suggest that it is only those species regarded as belonging to dry habitats (*Sabal mexicana* and *Washingtonia filifera*) that bear truly orthodox seeds, easy to store at low temperatures in the air-dry state. In contrast, the remaining species examined here are characteristic of comparatively moist habitats, and it appears that the seeds of none of them would be easy to preserve at low temperatures, largely because of their inability to withstand desiccation. Of the difficult seeds, some would be truly recalcitrant, while others may belong to an intermediate category in which a certain level of desiccation is tolerated but below which loss of viability occurs. Ellis et al. (1991) have demonstrated this type of behavior in seeds of at least one palm (*Elaeis guineensis*). The work presented here has not allowed clear differentiation between recalcitrant and intermediate seed storage behavior in the species examined, although the evidence available might point to the seeds of *A. wrightii* being intermediate and those of *O. cohune* being recalcitrant (Dickie et al. 1992).

The information on seed storage behavior generated in the present study, together with that compiled by Hong (1991), represents only 31 of a total of over 2,600 palm species. As well as being very small, the sample underrepresents species from moist habitats, which probably make up the great majority of palm species. Clearly, more work is needed to establish an adequate and unbiased database of palm seed storage characteristics, which could be used to assess the utility of *ex situ* storage in individual palm species conservation programs. In the meantime, it may be possible to suggest a rule of thumb whereby those species of definitely dry habitats are highly likely to bear seeds that are amenable to dry, cold storage, whereas those from relatively moist habitats are likely to be difficult or impossible to store. Even the latter group will contain species with intermediate seed storage behavior (Ellis et al.

(whole seeds were quartered) before and after drying at 103 ± 2° C for about 17 hours. Equilibrium relative humidities were measured using a Michel S-4020 dewpoint hygrometer.

Germination tests consisted of incubating seeds (and embryos in the case of *O. cohune*) on 1% (w/v) distilled water agar in either 9 cm polystyrene petri dishes or polystyrene sandwich boxes, in incubators maintained at 26° C, or fluctuating diurnally (33/19° C) with a 12-hour thermoperiod, illumination on a 12-hour photoperiod being provided by "warm-white" (Sylvania) fluorescent tubes. Incubation was continued until it was obvious that no further germination would or could occur. Palm seed germination is often quite protracted (Loomis 1958, Ellis et al. 1985) and in this work incubation periods varied from five weeks (*Washingtonia filifera*) to more than one year (*Acoelorraphe wrightii*). The tests were monitored at regular intervals, and germinated seeds (embryo extension >2 mm) were counted and removed. Whenever incipient drying out made it necessary, seeds were re-sown on fresh substrate.

In vitro techniques were also applied to embryos or seeds of *Orbignya cohune*, *Daemonorops verticillaris*, and two *Pinanga* species. After extraction from fruits, seeds were disinfected for 2 minutes in 70% ethanol, followed by 50% bleach for 20 minutes. They were then rinsed five times in sterile distilled water. Embryos were excised under aseptic conditions and individually cultured on 1 ml of MS (Murashige and Skoog 1962) medium containing 0.6% agar and 0.25% activated charcoal, in glass tubes sealed with polypropylene covers secured by rubber bands. For disinfection of excised embryos of *O. cohune*, a weaker (10–20%) solution of bleach was used. Cultures were incubated at 26° C in darkness. After 40 days, germinating seeds or embryos were transferred to fresh medium and incubated at 29° C with a 12-hour photoperiod.

For topographical tetrazolium staining of excised *Orbignya cohune* embryos, they were imbibed on paper towel wetted with distilled water for 24 hours at laboratory temperature (20–22° C), and then incubated in buffered 1% solution of 2,3,5-triphenyl tetrazolium chloride (Moore 1973) for 24 hours at 31° C in darkness. Embryos were scored as viable when they showed an overall even carmine red staining.

Results

Table 1 provides the results of the desiccation experiments. A full discussion of the results for eleven of the fourteen species examined here is presented in Dickie, Balick, and Linington (1992). Results for *Geonoma deversa*, *Jessenia bataua* and *Salacca zalacca* are added.

One species, *Acoelorraphe wrightii*, gave uncertain results. Germination of fresh seeds, as well as of seeds dried to <5% moisture content for 28 days, was erratic over a 15-month period. The viability of fresh seeds was 79% versus 17% viability for dried seeds. The greatly reduced rate of viability in the preserved seeds needs further quantification and study. However, it is felt that seed banking and cryopreservation will pose problems because of the above mentioned losses due to the drying process prior to freezing.

Discussion

Of the fourteen species of the Palmae examined so far in this investigation, the seeds of only two (*Sabal mexicana* and *Washingtonia filifera*) appear to be immediately amenable to seed bank storage or cryopreservation as means of *ex situ* conservation of genetic resources. This finding supports the commonly held view (J. Dransfield, pers. comm.) that considerable difficulties would be involved in attempts to conserve most palms *ex situ* as seeds. However, it is in contrast to data extracted from a recent literature search by Hong (1991), in which, of 21 palm species

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relatively frequent need for subculture and the fact that a single protocol is unlikely to be useful for diverse genotypes (Towill and Roos 1989).

In many areas of the Neotropics, entire palm populations have been destroyed; significant threats exist at the species level (e.g., *Attalea crassispatha*, see Henderson and Aubry 1989). If these palm resources are to be preserved for utilization and enjoyment by future generations, an increased level of action is needed in the conservation arena. For example, Brazil has a program to preserve the germplasm of economically important native palms, both *in situ* and *ex situ* (Coradin and Lleras 1988). This paper explores the possibility of *ex situ* conservation of members of the palm family in seed banks.

One group of plants, including several important tropical plantation species, bears seeds classified as recalcitrant (Roberts 1973). The main characteristic of such seeds is their inability to withstand any degree of desiccation and so they cannot be kept at sub-zero temperatures. Their lifespans are relatively short and cannot be extended by the methods presently employed to preserve germplasm in seed-banks or cryogenic stores.

Many palm species are propagated primarily by seed (Broschat and Donselman 1988) and, although Corner (1966, p. 183) maintains that palm seeds in general cannot withstand any degree of drying, the seeds of several species (e.g., date palm) are amenable to storage at freezing temperatures in the air-dry state (Al-Madeni and Tisserat 1986). However, there are also examples, such as coconut, of recalcitrant seed behavior among the Palmae (Chin and Roberts 1980), as well as at least one instance of apparently recalcitrant seeds (oil palm) being subsequently shown to be desiccation tolerant under certain conditions (Grout et al. 1983, Ellis et al. 1991). This paper reports on a study aimed at classifying seed storage behavior in a wide range of palm species, mostly having little or no previous work reported on them, with the aim of obtaining a reliable estimate of the proportion having seeds amenable to seed-banking or cryogenic storage.

Materials and Methods

Seeds of fourteen palm species were collected from sites in the United States, Central and South America, and Southeast Asia and sent to Wakehurst Place for investigation. The opportunistic nature of the collections meant that samples were often low in numbers of seeds, and the great distance they were transported meant that sometimes seeds were in poor condition on arrival at Wakehurst Place. Thus, the difficulties (including sporadic fruiting, obtaining export permits, and the time taken to transport seeds to the laboratory) inherent in working with tropical tree species precluded a rigorous experimental and quantitative analysis of all the palm seed samples received.

On arrival, fruits and seeds were stored moist in ventilated polythene bags at 16° C for up to one week before experimental treatments were begun. Where necessary, fleshy pericarps were removed by washing in tapwater. In *Orbignya cohune* the substantial, hard endocarps were removed by progressive and careful application of pressure in a large engineer's vice, with further pressure splitting the seed to permit removal of the embryonic axis.

In the control lots, seeds or embryos were set to germinate without any drying, i.e., at the relatively high moisture contents at which they arrived. Seeds or embryos were dried to low moisture content in a room at 15% relative humidity and 15° C in monolayers for periods up to four weeks. Throughout this paper, moisture contents are quoted on a percentage fresh weight basis. Measurements were made gravimetrically by weighing samples