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REPORT

TO THE

GOVERNMENT OF THE PHILIPPINES

on

THE CADANG-CADANG DISEASE OF COCONUT

AND

CITRUS VIRUS DISEASES

based on the work

of

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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ABSTRACT

Cadang-cadang, a virus-like disease of coconut, has been present in certain areas of the Philippines for at least 40 years. Knowledge about the disease resulting from more than 20 years of research by personnel of the Philippines Bureau of Plant Industry and the experts assigned by the Food and Agriculture Organization to the Bureau leads to the conclusion that the disease is not a nutritional deficiency, a soil toxicity, nor an air pollution problem. Recent attempts to transmit cadang-cadang mechanically and by "grafting" were unsuccessful. More than 15 species of insects were recently tested as potential vectors of cadang-cadang, but none of the test plants has developed the diagnostic symptoms of the disease. In previously reported work, more than 100 species were tried, also without success.

Cadang-cadang continues to spread, but at a lower rate than formerly. The incidence in a weed control plot of about 500 trees in Bay, Ligao, Albay rose from 1.06% when the trees were 19 years old in May 1962, to 19.9% in May 1971; the incidence in this grove increased during nine years in a linear fashion at the rate of about 2%/year. This linear rate of increase is probably reflected in a linear relationship of the incidence of cadang-cadang to age of trees. A completely satisfactory explanation for these linear relationships has not, however, been found.

Experimental weed control plots at the Guinobatan Experiment Station are under observation and should be continued for several years more. As yet there are no data to indicate a relationship between weeds in the plot and incidence of cadang-cadang.

There is good evidence that land on which a previous coconut crop has been destroyed by cadang-cadang will grow a good crop of coconut trees for 20 or 30 years before the incidence of cadang-cadang becomes serious. Farmers are therefore advised to replant to coconut.

Research needs to be continued on the cadang-cadang disease to determine its etiology and its probable vector. There is also need to continue selection and breeding to find resistant varieties or strains, and for introduction of germ plasm from abroad.

Leaf mottling of citrus is a serious disease in some parts of the Philippines and a threat to the other parts where citrus is grown. Studies directed toward its eventual control should be continued at the Lipa Experiment Station and extended to other regions such as the Guinobatan and Davao Experiment Stations.

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Chapter 1

INTRODUCTION

In accordance with a request from the Government of the Philippines for further assistance in research on the cadang-cadang disease of coconut, the Food and Agriculture Organization of the United Nations under the United Nations Development Programme (Technical Assistance Sector) appointed Dr. W.C. Price as Virologist to work with the Philippines Bureau of Plant Industry. Dr. Price served in the Philippines under the Technical Assistance Sector from December 1966 through December 1970 and is presently continuing under a United Nations Special Fund Project. His terms of reference were: to assist and advise the Government of the Philippines in investigations of cadang-cadang of coconut through the following measures: 1) study of the disease etiology; 2) study of the role of arthropods in the spread of the disease; 3) study of effective means of control. His terms of reference were broadened in January 1969 to include supervision of progress on citrus virus research and to advise the Government on those programmes aimed at the study and control of citrus viruses.

FAO is greatly indebted to the many people who collaborated with the expert during the assignment and who offered their helpful advice and assistance, especially to Eliseo A. Carandang (Director of the Bureau of Plant Industry), Epitacio A. Lanuza (formerly Assistant Director for Research), Bernardo S. Castillo (formerly officer-in-charge, Office of the Assistant Director for Research), Vicente B. Arancillo (Assistant Director for Operations), Freddy D. Jolindon (Chief, Budget Officer), Buenaventura C. Adriano (Director, Region 4 of the Bureau), Aquilino S. Cordova (formerly Acting Superintendent, Guinobatan Experiment Station), Domingo B. Protacio (Acting Superintendent, Guinobatan Experiment Station), Avelino E. Bigornia (Supervising Agronomist), to Gil O. Pableo, Magin L. Retuerma, and Erlinda Rillo (who were the expert's counterparts), and especially to Pericles S. Subido (Senior Research Chemist of the Bureau of Plant Industry), and Carlos A. Calica (Supervising Plant Pathologist of the Bureau of Plant Industry) whose advice on numerous occasions was extremely helpful.

Chapter 2

BACKGROUND INFORMATION

The Bureau of Plant Industry has been involved since 1949 in investigations of the cadang-cadang disease of coconut (Cocos nucifera L.), a destructive disease that has killed more than 12 million coconut trees in the Bicol peninsula alone (Figures 1, 2). Investigations carried out by personnel of the Bureau at the Guinobatan Experiment Station have yielded many pertinent facts, which have contributed in major ways to an understanding of the nature of cadang-cadang even though they did not provide proof of the etiology of the disease. Unfortunately the details of many of these investigations have not been published but were presented from time to time in mimeographed and typewritten reports. They have been summarized in various review papers (see, for example, Calica & Bigornia 1953, Calica 1963, Cruz 1962, Kent 1953, and Price 1958).

At the request of the Philippine Government, the Food and Agriculture Organization sent R.S. Vasudeva to the Philippines from 2 May 1955 to 29 July 1955 to appraise the cadang-cadang disease, the mosaic disease of abaca, and the research being carried out on these two diseases. Ever since Dr. Vasudeva's brief visit, the FAO has maintained an expert in the Philippines to advise and assist the Government in research on cadang-cadang. The work of these experts has been summarized in respective reports to the Philippine Government (Holmes 1962, Maramorosch 1963, McWhorter 1959, Nagaraj 1967, Nitzany 1969, Price 1958) and more briefly in a paper presented in Jogjakarta, Indonesia in September 1968 (Price & Nitzany, 1969).

As a background to the work to be presented in this report, a summary of the knowledge about the cadang-cadang disease existing in December 1966 is given.

2.1 DISTINCTIVE SYMPTOMS

It is generally conceded that the diagnostic symptoms of the disease are of two kinds: 1) in early stages, the apical end-or blossom end-of the husk of the fruit loses its triangular shape, becoming rounded, and the husk becomes thinner than normal and encircled with dark brown or black scarifications; 2) the leaves, beginning with the third from the spear leaf, develop small bright yellow spots without brown centres, which appear olivaceous or water-soaked when seen by light reflected from the under side of the leaf (Figure 3) (Calica and Bigornia 1963A). Other symptoms of cadang-cadang, such as brittleness and reduction in size of leaves, dropping of leaves faster than new leaves are formed, are not diagnostic by themselves because they may also occur as a result of water-logging and other troubles. Nevertheless, it is important to consider these symptoms and others-such as the shape, the colour, and stunting of the crown-when diagnosing cadang-cadang.

2.2 DISSEMINATION AND SPREAD

Cadang-cadang was present as early as 1928 in a plantation on San Miguel Island. During the next 35 years, the disease spread from the northwest sector of the plantation to the southeast until, in 1962, only 80 healthy bearing palms of an original 250 000 remained. By 1966, cadang-cadang was known to occur in the region from southern Quezon province in the north to the northern portions of Samar in the south.

In this region in which cadang-cadang occurs, the disease is not limited by soil types (it occurs on more than 42 types) nor by elevation (it occurs on the mountain tops, in the valleys, and along the seashore). Nor is it restricted to a particular soil type within a plantation, generally killing most, if not all, the trees in the plantation. The spread within a plantation is completely random, there being no evidence of clumping so as to suggest spread from a diseased tree to an adjacent one (Bigornia et al. 1960).

The incidence of cadang-cadang increased sharply in all four provinces of the Bicol peninsula between 1951 and 1952. Thereafter the changes in incidence were different in the four provinces. Between 1951 and 1957, the increase in incidence was 2.8-fold in Sorsogon, 2.9-fold in Albay, 4.8-fold in Camarines Norte, and 12.5-fold in Camarines Sur (Bigornia et al. 1960).

2.3 INCIDENCE IN TREES OF DIFFERENT AGES

At one time, it was believed that cadang-cadang did not affect trees before they flowered (Reinking 1950). It is now known that trees as young as 1 1/2 years from seed can develop the disease. The incidence in such young trees is, however, very low. Results of Sill, Bigornia, and Pacumbaba (1963) indicated that the incidence increased as the age of tree increased. In the trees studied by them, the incidence increased from 0.028 per cent in trees 1-5 years old to 63.8 percent in trees 50-60 years old.

2.4 EFFECT OF FERTILIZATION

A number of workers have carried out experiments in which fertilizer was applied to diseased trees to determine whether or not they would recover, and to healthy trees to see whether the disease could be prevented (De Leon & Bigornia 1953; Scharpenseel et al. 1955; Subido et al. 1960; Protacio (See Price 1958); Briones 1963). Fertilization was found to deepen the green of the leaves and to increase the yield of fruit in those trees that set fruit, but in no case did it induce recovery or prevent the disease. In most instances, fertilization hastened the decline of the diseased tree. In the work of Subido et al. the highest incidence of disease was in trees that received a complete fertilizer, including trace elements. The results of the work on fertilization suggest that cadang-cadang is not a nutrient-deficiency disease.

2.5 EXPERIMENTS ON TRANSMISSION

In a series of papers published from 1947 to 1963, Celino (1947, 1963) claimed to have transmitted cadang-cadang from a diseased coconut tree to a healthy one and to certain species of leguminous plants by mechanical inoculation. Transmission by mechanical means was also claimed by del Rosario and Quiaoit (1962) but the incubation period of the presumed virus in their inoculated seedlings was significantly different from that reported by Celino. None of these reports can be considered valid for the reason that other workers have not been able to reproduce them when using identical methods of inoculation, and other methods in addition.

Early attempts at the Guinobatan Experiment Station to transmit cadang-cadang were made with techniques that have suceeded with many virus diseases of annual and perennial plants - pin pricking into seedling coconut leaves in the presence of sap from a diseased palm, rubbing the leaves with sap both in the presence of Carborundum and without it - but to no avail. More than 2 000 seedlings were involved in these mechanical transmission

trials, as were also a number of seedlings of various annual and perennial plants. Among those who have tried these methods and failed are Calica, Holmes, McWhorter, Maramorosch, Nagaraj, Price, and Sill. These failures to transmit cadang-cadang do not prove that it is not an infectious disease - or even that it is not mechanically transmissible - but do suggest that it is not likely to be transmitted by ordinary methods of inoculation. Attempts to transmit cadang-cadang by means of insects have proved to be equally futile. During the years from 1949 to the present, A.E. Bigornia and associates have carried out extensive tests with potential insect vectors - more than 100 species being tested - but not a single experimental transmission has been demonstrated. Transmission of a "cadang-cadang-like disease" by Aphis gossypii Glover has been claimed by Protacio (1965); there is considerable doubt that the symptoms reported have any relation to cadang-cadang.

2.6 POSSIBLE RELATIONSHIP OF WEEDS TO CADANG-CADANG

Certain observations suggested to Holmes (1961, 1961A) that cadang-cadang spreads from weeds to coconut. Consequently in 1962 A.E. Bigornia started some experiments to determine the relationship of weed control to incidence of cadang-cadang. There was no significant difference in incidence of cadang-cadang among the various treatments in three plots with a total of about 1 400 trees after five years when the experiments had to be terminated for lack of funds. The incidence of disease in all three plots was, however, very low so that undue significance should not be attached to the results. A fourth plot, started in 1967, is still under observation.

2.7 EXPERIMENTAL REPLANTING

Experimental replanting of a 1.25 hectare grove in which the trees had been killed by cadang-cadang was started in 1954 by C.A. Calica, A.E. Bigornia, and G.O. Pableo. As of December 1959 the trees were growing well with an incidence of about 2 per cent.

In November 1957, D.B. Protacio (manuscript submitted for publication) began experimental replanting in an area in which there had been a high incidence of cadang-cadang and adjacent to which, on the north and south, diseased trees are present today. A replicated latin square arrangement was used for five different treatments. Twelve years after replanting, only one tree had come down with cadang-cadang.

The results of these various replantings suggest the following: 1) coconut trees will grow well in soil in which old coconut trees that developed cadang-cadang had been growing for years; 2) young trees were not infected by placing around their roots soil taken from around trees in an advanced stage of disease, chopped leaves, roots, and trunks from diseased trees, and certain fungi and nematodes. They lead to the conclusion that soil in a disease-present area does not contain an infectious agent or a toxic element capable of causing cadang-cadang and is not necessarily deficient in a nutrient needed for normal growth of trees.

2.8 SELECTION AND BREEDING

A long-range selection and breeding programme, designed to obtain a strain of coconut resistant to cadang-cadang was started by A.E. Bigornia in 1959 (Bigornia & Infante, 1965). Obviously, there has been an insufficient time lapse for significant results to have been obtained from this programme. It is hoped that the project can be continued long enough for such results to be secured.

2.9 THE PROBLEM

The knowledge about cadang-cadang available to the expert when he began his assignment in December 1966 - knowledge briefly reviewed in the preceding pages - leads almost without question to the conclusions that 1) cadang-cadang is neither a soil deficiency nor a soil toxicity problem, 2) it is not an air pollution problem, 3) it is, by elimination, an infectious disease, and 4) since no nematode, fungus, bacterium, or other microorganism can be found to be associated with the disease, it most probably is a virus or virus-like disease. It remained therefore to obtain evidence for the transmission of the disease, to determine the reservoir of infection, and to learn as much as possible about the effects of the disease on the cocoonut palm.

Chapter 3

CURRENT RESEARCH ON CADANG-CADANG

3.1 ATTEMPTS TO TRANSMIT BY MECHANICAL INOCULATION

Transmission of the lethal yellowing disease of coconut in Florida by a special technique of mechanical inoculation (Price et al.1967) gave hope that this special technique might work also for cadang-cadang. Consequently, special efforts were made to transmit cadang-cadang by means of new mechanical methods of inoculation. Unfortunately, none of the methods tried have succeeded.

The technique used successfully in Florida was to take inoculum from necrotic tissue and some of the adjacent tissue, to grind it in a mortar in the presence of enough tris-HCl buffer at pH 7.2—50 ml of 0.2M tris (hydroxymethyl) aminomethane + 41.2 ml of 0.2M HCl + 108.6 ml of distilled water, to form a slurry to which a little Carborundum was added, and to rub the slurry over the basal portion of the spear leaf of a young seedling. The inoculated leaf was rinsed with water, refolded, encased between the next two oldest leaves, and tied in place. The same technique was used for inoculating seedlings with cadang-cadang inoculum.

Inoculum was taken from trees in a medium or advanced stage of cadang-cadang, either from leaves with a large number of diagnostic spots or from unopened spathes. Very young spathes, 2 to 3 inches long, from diseased trees can be found in which the outer sheath appears more or less normal but the inner one shows patches of brown necrosis. Unopened inflorescences 6 to 8 inches long often contain a spadix that is partially necrotic. Attempts to isolate a fungus or a bacterium from the necrotic areas of such sheaths and spadices have been negative, suggesting that the necrosis is not the result of microbial activity. Necrotic tissue from such sheaths and spadices together with some of the adjacent normal appearing tissue was ground in a mortar in the presence of buffer to be used as inoculum. Leaves used as a source of inoculum were folded and shaved with a razor blade to obtain fine shavings that were then ground in a mortar in the presence of buffer. In some experiments fresh tissues were used; in others, the tissues were frozen before being ground.

In addition to tris-HCl buffer at pH 7.2, the following buffers were used: 1) 0.01M potassium phosphate plus 0.01M sodium diethyldithiocarbamate; 2) 0.5M tris-HCl + 0.02M ethylenediaminetetraacetic acid (EDTA); 0.075M boric acid at various dilutions; 3) 0.1M potassium phosphate at pH 7.4; 4) No.3 + 0.1M sodium diethyldithiocarbamate; 5) No. 3 + 1% sodium sulfite; 6) 5% polyethylene glycol MW 20,000; 7) 5% polyvinylpyrralidone MW 40,000; 8) No. 7 + 1% sodium sulfite; 9) 20% sucrose; 10) No. 8 plus activated charcoal; 11) 0.75% NaCl; 12) bentonite 25 mg/ml + 1% $KHPO_4$; 13) 0.01M tris HCl pH 7.4 + 0.01M $MgSO_4$ + 0.015M mercaptoethanol; 14) 0.03M phosphate buffer pH 8 + 0.01M sodium diethyldithiocarbamate; 15) 0.01M tris-HCl + 0.01M $MgSO_4$ + 0.015M 2-mercaptoethanol + bentonite; 16) No. 1 + bentonite; 17) No. 1 + 0.05 g/l sodium sulfite; 18) tris-HCl at pH 8; and 19) distilled water.

In some cases, inoculum was prepared by centrifuging extracts of tissue alternately at low speed (5 000 rpm) and high speed (40 000 rpm) and taking up the final pellet in water and buffer. It was hoped that whatever virus might have been present in the tissues would be concentrated by the centrifugation.

In each of many experiments involving the above mentioned buffers, six cocoonut seedlings about 9 months old were inoculated and six were rubbed with water as controls. The stipules were cut from the outer fronds so as to expose the base of the spear leaf, which was then rubbed with the inoculum on a gauze pad (Figure 4). Usually Carborundum was added to the inoculum. More than 700 coconut seedlings have been mechanically inoculated with the inocula

and procedures outlined above. In the same experiments, or in separate experiments, other species of plants were inoculated by the rubbing method. Among these are the following: Pandanus coplandii Merrill (PANDANACEAE); Zea mays L., Setaria palmifolia Stapf., Saccharum officinarum L. (GRAMINEAE); Elaeis guineensis Jacq., Livistona rotundifolia (Lam) Mart. (PALMACEAE); Gomphrena globosa L. (AMARANTHACEAE); Brassica pekinensis Rupr., B. oleracea L. (CRUCIFERAE); Cassia alata L., Vigna sinensis Savi., Phaseolus vulgaris L. (LEGUMINOSAE); Gossypium arboreum L. (MALVACEAE); Carica papaya L. (CARICACEAE); Stachytarpheta jamaicensis (L) Vahl. (VERBENACEAE); Lycopersicon esculentum Mill., Solanum melongena L., Capsicum frutescens L., Nicotiana tabacum L., N. glutinosa L. (SOLANACEAE); Cucumis melo L. (CUCURBITACEAE); Elephantopus mollis HBK, Ageratum conysoides L., and Lactuca sativa L. (COMPOSITAE).

The African oil palm was used particularly because in the Guinobatan area it develops a disease very similar to cadang-cadang and seems to be even more susceptible than the coconut palm.

Another method of inoculation used was to make inicisions into the blade and petiole of a leaf with a knife contaminated with sap of a leaf from a diseased palm. Forty test plants were inoculated by this method.

None of the test plants developed symptoms indicative of infection. Thus, it can be concluded that cadang-cadang was not transmitted to coconut or any of the other species by the mechanical procedures employed.

3.2 ATTEMPTS TO TRANSMIT BY GRAFTING

Successful grafting of monocotyledonous plants has not been accomplished. Yet McWhorter & Allen (1964) succeeded in transmitting the virus of lily stripe disease by grafting a piece of tissue from a diseased plant into a healthy one; they did not claim that a true union of tissues was obtained but there must have been enough of a union for the virus to pass from scion to stock. In view of this success, it seemed worthwhile to try several methods of grafting tissue from a diseased coconut palm into a healthy coconut seedling in hopes that the infectious agend would pass from the diseased tissue to the healthy. Actually, Calica in 1957 (unpublished work) grafted the tip end of fronds from a diseased coconut tree into the tip of fronds of healthy coconut and African oil palm seedlings. The scions lived for several weeks in plants kept in a moist chamber but did not transmit cadang-cadang.

One method of grafting used in the present work was to cut pieces of leaf 1/2 inch wide and 1 1/2 inches long, insert them into slits cut into the base of a petiole, and bind them into place with strips of plastic. Six coconut seedlings were inoculated in this way in each experiment.

Another method of grafting was that used by Schwarz (1968) and Cohen (1971) to transmit citrus tristeza virus. The first technique (after McWhorter and Allen) was to take pinnae from the 5th to the 18th frond, so as to have pinnae of various widths to match those of the test plants; a leaflet from the source plant was placed on top of a leaflet of the test plant, and a cut at either a 45° angle or 90° angle was made across the two leaves, serving both of them. The base of the cut off diseased leaf was then matched to the test leaf and bound in place with Scotch tape, or masking tape in some experiments. Then all but a 2-inch length of the diseased leaf was cut off, leaving the 2 inches attached to the test plant. Three adjacent leaflets on each test plant were enclosed in a plastic bag to prevent drying out. Such grafts lived for about one week and then died.

The second technique (after Cohen) was to place a leaflet from a diseased tree over a leaflet of the test seedling and then cut out a small triangular piece from each leaflet. The triangular piece from the diseased leaf was fitted into the triangular opening of the test leaf and bound in place. About 80 such grafts were made into each test seedling, dis-

tributed over about four leaves. The diseased tissue usually lived for 4-8 days - a maximum of 15 days - and then died.

A third method was to scrape the epidermes from a portion of a petiole of a diseased palm and a healthy seedling and bind the two together.

A total of 54 test seedlings were inoculated by means of these techniques. None has developed symptoms of cadang-cadang thus far.

3.3 ATTEMPTS TO TRANSMIT BY INJECTION

Three methods of injecting coconut seedlings and other pest plants were used, with a hypodermic syringe, with glass tubing drawn to a point and allowed to remain in place after being inserted into a petiole of a test plant, and by a "hypospray jet injector". The latter is an instrument used for mass immunization of humans; it develops tremendous pressure. Solutions for injection were prepared in various ways with the various buffers mentioned above; in some instances solutions concentrated by high-speed centrifugation were employed. A total of 108 plants were inoculated. None of them has thus far shown symptoms of cadang-cadang.

3.4 ATTEMPTS TO FIND AN INSECT VECTOR

Because of the distribution of cadang-cadang within various plantations and because of the observed pattern of spread in the epidemiological plots of Bigornia, there is good reason to believe that the disease is disseminated by an arthropod vector. The problem has been to find the vector. Bigornia and his associates have tested more than 100 species of insects as potential vectors, but without success. Most of the species tested were polyphagous, some of them even being capable of completing a life cycle on coconut. In the current research, some of the same species were re-tested, but other species - those not normally found feeding on the coconut palm and capable of existing on it for only short periods of time - are also being tested.

The basic procedure is the same as that used previously. Insects in groups of 50 or more were allowed an acquisition feeding period of 24, 48, 72, or 96 hours on leaves with the diagnostic symptoms of cadang-cadang and were then transferred to young coconut seedlings where they were allowed to feed until they died.

In the case of those species incapable of living for a long time on coconut, the basic procedure was modified. After an acquisition feeding period on detached leaves the insects were placed on a suitable host plant where they remained for 24 hours. They were then allowed an infection feeding period of 24 hours on a coconut seedling before being transferred back to the suitable host plant. Then they were transferred to a new coconut seedling. This procedure of alternating 24 hour periods on a suitable host plant and on coconut test plants was continued until all the insects died. Ten or more coconut seedlings were used as test plants in each experiment.

In some cases, each species was tested singly as a vector; in others, as many as five species were pooled on the same test plants. More than 100 test plants are now under observation. None has thus far developed symptoms of cadang-cadang.

The species of insects that have been tested as potential vectors in the current research are: Bemisia tabaci (Genn.), Chanitus graminium Fabr., Cicadella makilingensis Baker, C. spectra Dist., C. unimaculata (Sign.), Kamendaka philippinensis, K. tayabasensis Muir., Lamina flavescens Mel., Nephotettix apicalis Motsch., N. impicticeps Ishihara, Phaeocephalus sp. (prob. badius) (Muir.), Thrips sp., Typhlocibinae sp., Virgilia luzonensis Baker, and Zuegma valdezi (Muir.).

3.5 ATTEMPTS TO TRANSMIT BY ERIOPHYID MITES

At the request of the Philippine Government, FAO appointed Norton S. Wilson (Research Entomologist), to assist in the collection and taxonomy of eriophyid mites in the Bicol region, with reference to their possible role in vectoring cadang-cadang of coconut, and to train his counterparts in the technique of handling mites for transmission studies. Mr. Wilson spent about two months working with A. E. Bigornia at the Guinobatan Experiment Station in the autumn of 1970 (17 September-1 December). Working together, Wilson and Bigornia discovered seven or eight new species of eriophyid mites. One of the new species is a coconut feeder and belongs to a group of mites containing species that are known to vector viruses.

Eriophyid mites were allowed to feed on a diseased coconut tree as a source of inoculum and were transferred to test plants to determine whether or not they can transmit an infectious agent from the diseased coconut palm, large numbers of mites being used in each case. The following numbers of test plants in pots - and a comparable number of controls - were used: 77 of coconut; 32 of African oil palm; 15 of anahaw (Livistona rotundifolia (Lam.) Mart.; 70 of corn (Zea Mays L.); 62 of rice (Oryza sativa L); and 6 of palm grass (Setaria palmifolia Stapf.). Thus far none of the test plants has developed symptoms indicative of cadang-cadang. They are being kept under observation in a screenhouse.

It has been necessary to discontinue Bigornia's experiments with mites because of the unavailability of funds to support them. It is hoped that they can soon be resumed because of the possibility that one or another species of eriophyid mites can vector cadang-cadang.

3.6 EPIDEMIOLOGY

It is now known (Price & Bigornia, 1969) that the region infested by cadang-cadang extends from Infanta, Quezon Province, in the north of Guiuan, Samar, in the south. Certain areas near Infanta are heavily infested but the incidence between Infanta and the Bondoc peninsula seems to be rather low. A similar situation apparently exists in Samar, with an incidence of 10 - 30 percent in the peninsula below Salcedo and a fairly high incidence in the northern portion of Samar, but a low incidence between the two areas. In this connection, it should, however, be pointed out that no extensive surveys have been made in Samar except in the southeastern peninsula in 1968.

Bigornia et al. (1960) have studied the epidemiology of cadang-cadang in strip surveys and in epidemiological plots. Price & Bigornia (1969) extended these studies by making strip surveys in Albay and Camarines Sur Provinces. In these two provinces, the incidence rose from 19% and 5%, respectively, in 1951 to highs of 54% and 61% in 1959 and then dropped to 9.6% and 31.8% in 1967; the incidence given are for trees 20 years old or older; the incidences in trees less than 20 years old are much lower, being 0.6% and 3.0%, respectively, in 1967. Additional strip surveys were made in Sorsogon province in May 1971. Of 35 416 trees 25 years old or older that were examined, 3 217 - about 9.1% - were affected by cadang-cadang. In this province, the incidence had reached a maximum of 40.3% in 1956 (Bigornia et al. 1960). It is apparent that there has been a substantial decrease in incidence in Sorsogon, just as there has been in Albay and Camarines Sur. Of 42 160 trees 5 to 25 years old examined in the same survey, only 465 or 1.1% were diseased with cadang-cadang.

One of Bigornia's weed control plots - Plot 1 at Bay, Ligao, Albay - was originally in a grove in which all the trees were killed by cadang-cadang. It was replanted with more than 590 coconut seedlings in 1953. A photograph of one edge of the plot taken in 1957 has been published (Fig. 7 in Price 1958). The incidence of cadang-cadang in 472 trees in this grove can be calculated from Table 1 of Bigornia (1969). The incidence in all trees in the grove in May 1971 was determined by the expert's counterparts. The data are in Table 1. Nine years after the grove was replanted, the incidence was only 1.06%. It then rose gradually

to 9.75 percent during the next five years. By 1971, when the trees were 28 years old, the incidence had risen to 19.9 percent. This slow increase in incidence seems to be typical of coconut groves in the vicinity of Guinobatan, Albay. A photograph of a grove that was destroyed by cadang-cadang and subsequently replanted to coconut is in Figure 5.

According to Sill et al. (1963), cadang-cadang is primarily a disease of old trees, the incidence increasing steadily as the trees grow old. Price and Bigornia (1969) plotted the data of Sill et al. as a scatter diagram and found that the regression of incidence on age of tree was accounted for almost entirely by a linear trend, the deviations from linear being not significant; the slope of the regression line indicated that there was, on the average, a 1.04 percent increase in incidence per year of age of tree. This relationship between incidence and age has not been satisfactorily accounted for. In order to arrive at a possible explanation for it, consideration will be given to the spread of cadang-cadang in epidemiological plots and as determined by roadside surveys.

The manner of spread of cadang-cadang in epidemiological plots and along roadsides has been examined by Holmes (1961) whose explanation is that the disease does not spread from tree to tree but from a weed reservoir of an infectious agent, that the size of this reservoir varies from plot to plot, and that the reservoir plants are clumped in the plot. Price & Bigornia (1969) showed, however, that there was no significant clumping of diseased trees within plots and that the distribution was strictly random.

The data from the roadside surveys of Bigornia et al. (1960) from 1951 to 1957 are plotted as a scatter diagram in Figure 6, and a straight line with a slope of 5.1%/year was fitted to the points. Except perhaps for the sharp rise in incidence from 1951 to 1952, the data do not depart significantly from a linear trend. It is possible that the sharp increase in incidence from 1951 to 1952 is due to the survey crew having learned by experience how to diagnose trees in early stages of the disease. Between 1957 and 1959 there was no increase in incidence, and from 1959 to the present there has been a substantial decrease (Price & Bigornia, 1969).

The data from Table 1 may also be plotted as a scatter diagram (Fig. 7). From the figure it can be seen that in the plot at Bay, Ligao, the incidence rose in a linear fashion from 1962 to 1971 at a rate of 0.164%/month or 1.97%/year. The regression was highly significant ($F = 150$).

If the spread of cadang-cadang in a grove occurs from year to year at a linear rate, as indicated in the preceding two paragraphs, then it is not surprizing that there is a linear relation between incidence and age of tree. The problem is how to account for the linear rate of spread. A possible explanation is as follows:

Let it be assumed that in a grove of 1 000 or more healthy trees there is a small, but fixed, probability - say 0.01 - that a tree will become diseased each year. On the further assumptions that cadang-cadang is an infectious disease transmitted by an insect vector and that the source of inoculum lies outside the grove in the beginning, the increase in incidence for the first few years will be linear. Since a diseased tree will live for 8 to 15 years - about 10 years on the average - there will come a time when the increase in incidence in the grove will be offset by the death of trees infected 8 to 15 years previously. When this time is reached, the incidence in the grove will remain constant, or even decrease, unless the probability of a tree in the grove becoming diseased is increased. The increase in probability required for a continued linear increase in incidence can be accounted for on the assumption that the diseased trees in the grove furnish an increasing reservoir of an infectious agent from which the disease can spread to other trees in the grove.

The explanation given above is not intended as proof that cadang-cadang spreads from

tree to tree but merely that its rate of spread can possibly be accounted for on this basis. One difficulty is that the explanation fails to account for the very low incidence observed in trees less than 20-25 years old, it being 3.02% in Camarines Sur and 0.62% in Albay in 1967 (Price & Bigornia, 1969) and 1.10% in Sorsogon in 1971. Nor does the explanation take into consideration the variability resulting from environmental factors of a theoretical vector population. If there is an arthropod vector, it must be either very rare or very inefficient, or both, to be involved in such a low rate of spread.

Bigornia (1969) has pointed out that in weed control plots in five years of observation there was no correlation between the presence or absence of weeds in the sub-plots and the increase in incidence of cadang-cadang. This fact can be illustrated by the Bay, Ligao, plot. The incidence in each sub-plot in 1962 was about 1% or less. By 1967 it had risen to 9.75% in the plot as a whole (Table 1), with incidences in the sub-plots as follows: clean cultivation, 10.6%; weedicide, 7.8%; insecticide, 13.3%; cover crop, 7.5%; and control, 9.3%. It is apparent that the presence of weeds in the sub-plots during a five year period did not contribute substantially to the incidence of cadang-cadang. It is also apparent that the use of insecticides to control possible vectors did not prevent the spread of the disease in a sub-plot of 98 trees.

3.7 EXPERIMENTAL WEED CONTROL

Because Bigornia's weed control plots outside the Guinobatan Experiment Station had to be abandoned, it was decided to establish in 1967 a new plot inside the station. The trees involved were mostly of the Tambolilid variety planted in 1958. A randomized design of three one-half-hectare blocks was provided for six treatments as follows: 1) clean cultivation; 2) application of weedicide sprays; 3) cover cropping to Pueraria sp.; 4) application of insecticide sprays; 5) underplanting with abaca; and 6) no weed control. The treatment of underplanting with abaca was included because of the report by Nagaraj (1967) that there was a low rate of spread of cadang-cadang in coconut gardens in which abaca, banana, or coffee was so densely underplanted that dicotyledonous weeds were eliminated. In the beginning, in March 1967, there were only five diseased trees in the entire plot. By 1971 only seven trees were affected by cadang-cadang. The incidence is thus too low to judge the effectiveness of the various treatments in preventing the development of the disease.

3.8 ANATOMICAL STUDIES

To gain additional insight into the nature of cadang-cadang, Mrs. Erlinda Rillo and the expert carried out anatomical studies with leaves from diseased plants and from healthy plants for comparison. Unsatisfactory sections were obtained with the paraffin method of embedding after fixation in FAA, Craf III, and Karpechenko fixatives. Good preparations were obtained by cutting fresh sections with a sliding microtome, fixing them in FAA, and staining in fast green and safranin. Because the coconut leaf is very tough, it was not possible to cut fresh sections thinner than 8 microns.

The normal coconut leaf has been described by Tomlinson (1961); a camera lucida drawing of a cross section of a normal leaf is in Figure 8A. Both the adaxial and abaxial epidermis consists of a single layer of thickly cuticularized cells. Below the adaxial epidermis are two layers of hypodermal cells; only one broken layer of hypodermal cells lies above the abaxial epidermis. Below the adaxial hypodermal cells are four to five layers of closely packed chloroplasts-filled mesophyll (palisade) cells; they are regularly arranged, more or less in tier formation. The chloroplasts are distinct, discrete bodies and are normally aligned along the periphery of the cells. Five or more small vascular bundles alternate with large veins in the palisade tiers, the large veins being usually attached to the lower and upper hypodermis by a few sclerenchymatous cells or fibres. Fibre cells are regularly interspersed in the second layer of the hypodermis and extend to the first palisade layer.

The large bundles consist of tracheids and vessels, three or four phloem elements, and a few fibre cells. The phloem elements occur towards the abaxial epidermis and the xylem elements toward the adaxial. Both small and large veins are encircled by a narrow band (1-2 layers) of bundle sheath fibres. The abaxial hypodermis (water cells) generally consists of only one broken layer; the hypodermal cells are often absent. Stomata are regularly distributed in the abaxial epidermis. Lastly, dark brown, short depressions containing tannin occur regularly in the abaxial epidermis.

As seen in a cross section of a diseased leaf (Figure 8B), the cells are present in the same number and arrangement as in the healthy. Changes occur in the green portion of the leaf as well as in the yellow spots. The changes are as follows: 1) the blade of the leaf is thinner, especially so as the disease advances, the reduced thickness being accounted for by a smaller size of all types of cells; 2) mesophyll cells are not rectangular, but rounded, and are irregularly arranged rather than in tiers; 3) chloroplasts begin to disintegrate in the early stage of the disease and appear to form a single mass inside the cells; in the advanced stage they are completely obliterated; cells with disintegrating chloroplasts become red or purple when stained with fast green and safranin, suggesting that they are necrotic, 4) disintegrated chloroplasts give the cell contents a gray green or purple granular appearance in stained sections; in some cases there is evidence of spherical chloroplasts but those chloroplasts that remain appear in compact groups; 5) nuclei appear to be absent; those that remain visible are indistinct; 6) elements of the vascular bundles are present but generally reduced in size; the bundle itself appears to be round instead of pear-shaped.

Some of Mrs. Rillo's prepared slides were examined by Dr. Lynne L. Hoefert (USDA-ARS, Sugarbeet Investigations). She pointed out that the small size of the cells observed in the leaf of the diseased palm is likely the result of incomplete or arrested development - the tissues are hypoplastic or immature or juvenile, a fact that accounts for the irregular arrangement of cells. The symptoms resemble those of a mosaic disease.

McWhorter (1959) pointed out that there is only one kind of leaf spot characteristic of cadang-cadang; it appears yellow when viewed by transmitted light and water-soaked or olivaceous when viewed by light reflected from the under surface. He stated that such spots are due to what is commonly referred to as white cell necrosis.

Cells in sections cut through a green portion of a diseased leaf generally absorbed stains in the same manner as those in a healthy leaf, but some developed a reddish-purple tinge, indicating the beginning of necrosis. Even in the green portion of the leaf, the cells were reduced in size. The cuticle in both types of sections stained light red. The fibres regularly distributed in the abaxial hypodermis, including the fibre sheath of the vascular bundle, stained dark red. The palisade cells, chloroplasts, and nuclei stained green. The xylem vessels stained very light red whereas the phloem elements stained green. Cells in the characteristic yellow spots are evidently without chloroplasts. Complete disintegration must have taken place in order for the solid mass to stain deep red.

3.9 CHARACTERIZATION OF THE DIAGNOSTIC SPOT

One of the controversial aspects of cadang-cadang is its diagnosis. Although there is fairly general agreement that the occurrence of a characteristic yellow spot on the pinnae and the rounding and scarification of the husks is sufficient for diagnosis, there are those who doubt the diagnostic aspect of these symptoms - yet they offer no diagnostic criteria of their own choosing. In any case, it seems that a thorough characterization of the specific yellow spots, as one of the diagnostic symptoms, would be very much desired.

As mentioned above, McWhorter (1959) stated that there is only one kind of spot diagnotic for cadang-cadang, the one designated as white cell necrosis. Nagaraj et al. (1965) described the various kinds of spots to be seen on coconut leaves in certain parts of the Philippines. The spots they believe to be specific for cadang-cadang are described as follows:

"watersoaked spots, small, olivaceous, irregular, margins distinct from adjoining lamina, 1/8 to 1/2 mm in diameter, sometimes forming streaks.". The authors reported the number of trees examined and the number with each kind of spot but gave no data on measurement of size and number of spot.

It is generally recognized, of course, that there are various fungi that cause spots on the leaves of coconut, and that mites and insects initiate yellow spots while feeding on coconut leaves. In addition, there are tiny yellow spots of unknown etiology to be found on the leaves of both healthy and diseased coconut trees. The tiny yellow spots of unknown etiology are apparently the same as those designated as Type 1 by Nagaraj et al. (1965) except that they are not quite so severely restricted in size, as will be shown later. They differ from the diagnostic spots, Type 4 of Nagaraj et al., in several ways.

A principal difference is that the tiny yellow spots of apparently healthy trees do not, with rare exceptions, appear olivaceous when viewed by light reflected from the under surface of the leaf. The diagnostic spots do appear olivaceous by light reflected from the under surface of freshly harvested leaves and from both surfaces after the leaf is allowed to dry out for an hour or two.

Counts were made to determine the differences in numbers of spots on leaves of healthy and diseased palms and among stages of the disease. Since casual observation had indicated that parts of leaves towards the sun have more spots than those away from the sun, fronds were taken from the sunny side of the tree and from the shady side of the tree. Also, since the pinnae on a coconut tree are folded along the midrib, a record was kept of the side of the pinnae toward the sun and that away from the sun. Counts were made under a dissecting microscope of all the spots in the microscopic field near the base, in the middle, and near the tip of each pinna. The total area in the three fields is 84.8 mm^2. Counts were made on the pinnae from fronds 3 to 17 (counting from the spear leaf) of three healthy trees and three in each of three stages of the disease - early, medium, and late. The data are summarized in Table 2.

Although the data have not been analyzed statistically, several conclusions seem warranted: 1) the side of the pinna toward the sun has more spots than the side away from the sun; 2) fronds from the sunny side of the tree have more spots than those from the shady side; 3) the number of spots increases in a linear fashion from the 4th to the 15th leaf (Figure 9); 4) the numbers of spots increases as the disease advances. The slopes of the lines in Figure 9 are: Healthy, 5.72; early, 9.17; medium, 10.8; and late, 14.54.

A study was made of the sized of the tiny yellow spots that appear on apparently healthy trees and of the diagnostic spots of cadang-cadang. Samples were collected from Infanta, Quezon, the provinces of Masbate and Davao, and the southeastern tip of Samar as well as from Albany, Camarines Sur, and Sorsogon provinces. The diameter of each of 100 spots on a single leaf was measured with an ocular micrometer in a dissecting microscope. The means of these diameters were then subjected to analysis of variance.

Spots on otherwise healthy plants varied in diameter from a minimum of approximately 0.05 mm to approximately 0.90 mm; the diagnostic spots varied from about 0.05 mm to about 1.5 mm. The mean diameters of the 100 spots counted on each leaf varied from about 0.09 to about 0.31 mm on the healthy plants and from .09 to 1.06 mm on the diseased. In the case of both healthy and diseased palms, individual trees varied significantly in the average size of spots on their leaves. The diameter of spots also varied according to the location from which the trees were obtained but the variation was not significantly greater than that between trees of the same location. In a special experiment with diseased trees in which the trees were selected according to the stage of the disease, the variation among trees of different stages was not significantly different from that among trees of the same stage.

The greatest variation in size of spots was that due to age of the leaf (its position on the tree) for both healthy and diseased trees. The size of spots increased linearly with the leaf position, counting from the third leaf from the spear leaf. The variation due to non-linear factors was not significant. The regression coefficients for the 22 healthy trees varied from 0.0018 to 0.025 with a mean of 0.00985; those for the 53 diseased trees varied from 0.0034 to 0.082 with a mean of 0.0251. Thus, the size of spots in the diseased trees increased about 2.5 times as rapidly as that in the healthy trees. A test for homogeneity of the regression coefficients indicated a significant variation among trees; the variation in slope among trees of different stages of disease was not significantly greater than that among trees of the same stage.

It is concluded that the yellow spots without brown centres and with irregular but distinct margins on leaves of diseased trees, not only are much more numerous than those on apparently healthy trees, but also become larger in diameter; they also appear olivaceous in reflected light - even in a very young leaf - whereas those on apparently healthy trees do not.

Chapter 4

EXPERIMENT ON A LEAF CURL DISEASE OF TOMATO

The attention of the expert was called to a leaf curl disease of tomato that has been a major problem to tomato growers in the Province of Albay since 1968. Although the pathogenic agent of this disease is transmitted from tomato to tomato by the whitefly Bemisia tabaci (Genn.), it is different from that which causes tobacco leaf curl and that which causes tomato yellow leaf curl of Israel. Investigation of the disease - when time could be spared from the investigations of cadang-cadang was undertaken for three reasons: 1) because an understanding of the disease would be of value to the tomato farmers of the Bicol region, 2) to determine whether it might have a relationship to cadang-cadang, and 3) to provide the expert's counterparts with experience in investigating a virus-like disease of plants. A report of the investigations was given at the annual meeting of the Philippine Phytopathological Society held in Zamboanga City, 3-5 May 1971, and a paper has been submitted for publication in Philippine Phytopathology (Retuerma et al. 1971).

The disease is characterized by upward curling or cupping of leaves, by yellowing of the foliage, by streaking of immature fruit with yellow blotches, and by general stunting and reduced yields. It was readily transmitted to tomato by grafting and by the whitefly, but was not transmitted by either means to tobacco, N. glutinosa, eggplant, Datura sp., or a number of other herbaceous plants, nor was it transmitted back to tomato, from these herbaceous plants, indicating that they are not symptomless carriers. It was not transmitted to coconut by the whitefly. It appears not to be related to cadang-cadang.

A minimum of two whiteflies transmitted the causal agent after suitable acquisition and infection feeding periods. Maximum transmission was obtained with 10-20 whiteflies. The whitefly is capable of transmitting the causal agent after an acquisition-feeding period as short as one hour and during an infection-feeding period of only three minutes. It was not transmitted by mechanical means other than grafting. None of the varieties of tomato tested seemed to have resistance to the disease.

Chapter 5

VIRUS AND VIRUS-LIKE DISEASES IN THE PHILIPPINES

5.1 LEAF MOTTLING

The major virus disease problem confronting citrus growers in the Philippines is that known locally as leaf mottle yellows or leaf mottling (Martinez & Wallace 1967, 1968;) Salibe and Cortez 1968). The disease is probably the same as yellow shoot of China (Lin 1956), likubin of Taiwan (Matsumoto et al. 1961), phloem degeneration of Indonesia (Tidawidjaja et al. 1965), and dieback of India (Fraser & Singh 1968). There is good reason to believe that it is also the same disease as greening of South Africa (Oberholzer et al. 1965); both diseases are vectored by psyllids. The disease was present in Batangas, Laguna, and Quezon provinces as early as 1957, and it is now known to be in Cavite, Rizal, Albay, Camarines Norte, Camarines Sur, Mindoro Oriental, Negros Occidental, Cotabato, and Cebu provinces (Cortez & Celino 1971). Since much of the citrus grown in various parts of the Philippines was propagated from trees growing in Batangas province, it is likely that the greening pathogen is present in each region where citrus is grown, but is widespread in only a few provinces. More than a million citrus trees have been killed by greening in the Philippines. In 83 representative orchards in Batangas province, production of citrus rose between 1955 and 1960 to a peak of 11 680 000 kg, then declined to 1 000 000 kg by 1968 (Martinez et al. 197).

Largely because of the damage to citrus caused by leaf mottle yellows in Batangas province - and at the request of the Philippine government - FAO has provided experts and a certain amount of equipment for the Lipa Experiment Station. The Philippine workers at the Station have continued research on this important disease and its psyllid vector. A very important finding is that budsticks of citrus can be freed of the pathogen by immersing them in a 1 000 ppm solution of various tetracycline compounds for about 25 minutes (Martinez et al. 1970). The technique of treating budsticks with tetracycline solutions should allow the propagation of citrus budlings free of the greening pathogen for distribution to commercial growers.

Ecological studies on Diaphorina citri Kuway initiated by Catling (1968) have been continued. A study of the relationship of the flushing rhythm of citrus to the population dynamics of D. citri was begun at the Lipa, Guinobatan, and Davao Experiment Stations. Unfortunately, the financial difficulties of the Bureau of Plant Industry prevented this study from being continued long enough for significant data to be secured from all three stations. It is unfortunate that this study cannot be continued since it could very well yield important data by means of which better methods of controlling D. citri could be devised. There is reason to believe (Catling 1968) that large populations of D. citri are essential for rapid spread of leaf mottle yellows and, therefore, that effective means of reducing the population of this insect would permit the profitable production of citrus in areas where the disease is endemic.

Although leaf mottling is present in the Guinobatan area, it does not seem to be causing the damage here that it has caused in the Batangas area. Moreover, it is known that certain trees of Ladu mandarin have carried the pathogen for several years but they have not shown severe symptoms and have continued to grow fairly vigorously and to produce a good crop of fruit. Additional studies on leaf mottling in the Bicol region would be very useful to citrus growers in this and other regions.

The deep-well pump furnished by FAO to the Lipa Experiment Station has provided an irrigation system that makes possible such field studies as tolerance of various citrus cultivars to the leaf mottle yellows pathogen (Gonzales et al. 1971). Since there are indications that irrigated groves withstand the onslaughts of the disease better than non-irrigated ones, the irrigation system at the Lipa Experiment Station could be used at a future time to investigate such a possibility.

A BPI Citrus Budwood Certification Programme has been underway at the Lipa Experiment Station. Its purpose is to supply citrus budwood of various varieties, selected from vigorous, productive, true to type mother trees, and free of known viruses for use in propagating budlings for distribution to citrus growers. It is anticipated that this programme will be headquartered eventually on the island of Mindoro, in a region reported to be relatively free from greening and its vector. Such a programme, if properly carried out, should be very valuable to citrus growers in the Philippines. It should be accompanied by implementation of a quarantine programme to regulate the movement of citrus plants within the country.

5.2 TRISTEZA

Tristeza and its vector, Toxoptera citricidus Kirkaldy, seem to be ubiquitous in the Philippines. Moreover, the strain of virus that is ordinarily present in infected trees is relatively severe. It is fortunate that most of the varieties of citrus grown in the country are more or less tolerant of the ordinary strains of tristeza virus. Yet the West Indian lime (Citrus aurantifolia (Christm.) Swingle) is severely damaged by most of the ordinarily occurring strains. The calamondin (C. madurensis Loureiro), which is grown extensively throughout the country, tolerates most of the ordinarily occurring strains of tristeza virus, but even it is sometimes severely damaged by unusually severe strains. One approach to the control of tristeza in calamondin is cross-protection, i.e., to propagate from trees carrying a mild strain of tristeza virus, in order that the propagations will be protected from more severe strains being spread by the vector in citrus groves.

Studies were carried out at the Guinobatan Experiment Station (when time could be spared from investigating cadang-cadang) to learn more about the mechanism of spread of tristeza virus by T. citricidus. An abstract of a paper presented at the annual meeting of the Philippine Phytopathological Society in Iloilo City 5-8 May 1970 has been published (Retuerma & Price 1970). It was found that aphids allowed an acquisition feeding period of only a few seconds picked up tristeza virus from an infected leaf of West Indian lime and transmitted it to a healthy test plant. Aphids transmitted tristeza virus after an infection feeding period of only a few seconds. They also transmitted it in a total lapsed time of about 1 1/2 minutes from the beginning of an acquisition feeding period to the end of an infection feeding period. These results leave no doubt that tristeza virus is stylet borne. In a series of tests in which aphids were allowed an infection feeding period of 24 hours and then transferred to a healthy calamondin plant before being transferred to a succession of West Indian lime test plants, the aphids retained the virus for a maximum of 6 hours. It is doubtful that retention of the virus for only 6 hours is sufficient evidence to conclude that the virus is also circulative.

Chapter 6

CONCLUSIONS AND RECOMMENDATIONS

Vigorous attempts to reproduce the cadang-cadang disease by mechanical inoculation and by means of insects resulted in failures. The etiology of the disease therefore remains problematical. Additional studies of its epidemiology confirmed previous findings that its spread and distribution is similar to that of known infectious diseases rather than to nutritional, soil toxicity, or air pollution disorders. Re-evaluation of the known facts about cadang-cadang confirm the previously held conclusion that it is probably of virus or virus-like nature.

Study of the yellow spots appearing in leaves of trees affected by cadang-cadang indicates that the characteristic spots are, as previously reported by others, yellow when viewed by transmitted light, olivaceous or of a water-soaked appearance when viewed by light reflected from the undersurface of the leaf or the petiole, irregular in outline, without brown centres, and have margins distinct from the adjoining lamina. They range in size from 0.05 mm to 1.5 mm in diameter and in number from less than $4/mm^2$ to more than $200/m^2$. They increase both in size and number at a linear rate as the leaf grows older and are more numerous and larger in late stages of disease than in early stages. When taken in conjunction with other symptoms of cadang-cadang - such as rounding and scarification of the nuts, stunting and brittleness of leaves, and loss of lower leaves from the crown - they are considered to be highly diagnostic. Trees without such characteristic spots in their leaves should certainly not be diagnosed as affected by cadang-cadang. A cross-section through one of the characteristic spots reveals distinctive changes, including reduction in size of all types of cells, rounding and irregularity of arrangement of mesophyll cells, and disintegration and obliteration of chloroplasts and nuclei. The symptoms are reminiscent of those of a mosaic disease.

Cadang-cadang continues to spread in the provinces of the Bicol region, though perhaps at a rate considerably less than that obtaining during the fifties. In 1957, it was estimated that about half the trees in the Bicol peninsula (about 8 million trees) were affected by cadang-cadang. Today, a rough estimate of the number of diseased trees in the same area is 5 million. A part of this reduction in total number of diseased trees is due to the fact that many diseased trees have died and that newly planted trees have not grown old enough to show a high incidence of disease.

There is presently no evidence from experimental weed control plots that control of weeds, especially broad-leaved ones, will reduce the spread of cadang-cadang. These experiments are, however, in the early stages and should be watched carefully in future years.

It is recommended that farmers continue to replant devastated groves to coconut since it is very likely that these replanted groves will prove to be highly profitable, despite the probability that many of the trees will become diseased after reaching an age of 20-25 years.

In view of the value of the cocoonut palm to the Philippines in general and to the Bicol region in particular, it will be highly desirable to continue research on the cadang-cadang disease to ascertain its nature and to develop varieties of cocoonut that are immune or resistant. In this connection, the importation of germ plasm from other countries should be encouraged. The breeding and selection programme initiated by A.E. Bigornia should be continued for many years even though there is presently no assurance that the trees that have survived the onslaughts of cadang-cadang carry genes for resistance or immunity.

Although the failure of the whitefly *B. tabaci* to transmit the pathogenic agent of tomato leaf curl to cocoonut is good evidence that there is no relation between leaf curl and cadang-cadang, additional studies on leaf curl should be undertaken to learn something about its epidemiology. The disease is very important to the tomato growers of the Bicol area and perhaps other areas of the Philippines.

Greening - or leaf mottle yellows - remains an important problem for citrus growers of the Philippines. Work on this disease at the Lipa Experiment Station should be encouraged and expanded. It is recommended also that the work on this important disease include studies at the Guinobatan and Davao Experiment Stations. Additional information is needed on the distribution of the disease in the Philippines as a whole and on the spread of the disease and its effects in parts of the Philippines other than Batangas province.

Appendix 1

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Appendix 2

TABLES

Table 1.

Incidence of cadang-cadang in a grove at Bay, Ligao, Albay, which was replanted about 1943 after most of the old trees had been destroyed by cadang-cadang. Incidences in 472 trees from May 1962 to March 1967 from Bigornia (1969)

May 1962	Dec. 1962	June 1963	Dec. 1963	May 1964	May 1965	Dec. 1965	May 1966	Dec. 1966	March 1967	May 1971
%	%	%	%	%	%	%	%	%	%	%
1.06	1.91	3.39	5.72	8.26	8.90	9.53	9.75	9.75	9.75	19.9

Table 2.

Number of yellow spots in three microscopic fields - area 28.26 mm^2 - in the pinnae of fronds 4 to 17, counting from the spear leaf, of coconut trees in various stages of cadang-cadang and in healthy trees. Total area represented by each sum in the table is 1/186 mm^2

	Stage of Disease				
	Healthy	Early	Medium	Late	Total
Sunny Side of Frond					
Sunny Side of Pinna	197	327	350	449	1323
Shady Side of Pinna	148	219	234	325	926
Shady Side of Frond					
Sunny Side of Pinna	183	283	281	414	1161
Shady Side of Pinna	137	187	205	280	809
Total	665	1016	1070	1468	

Appendix 3

ILLUSTRATIONS

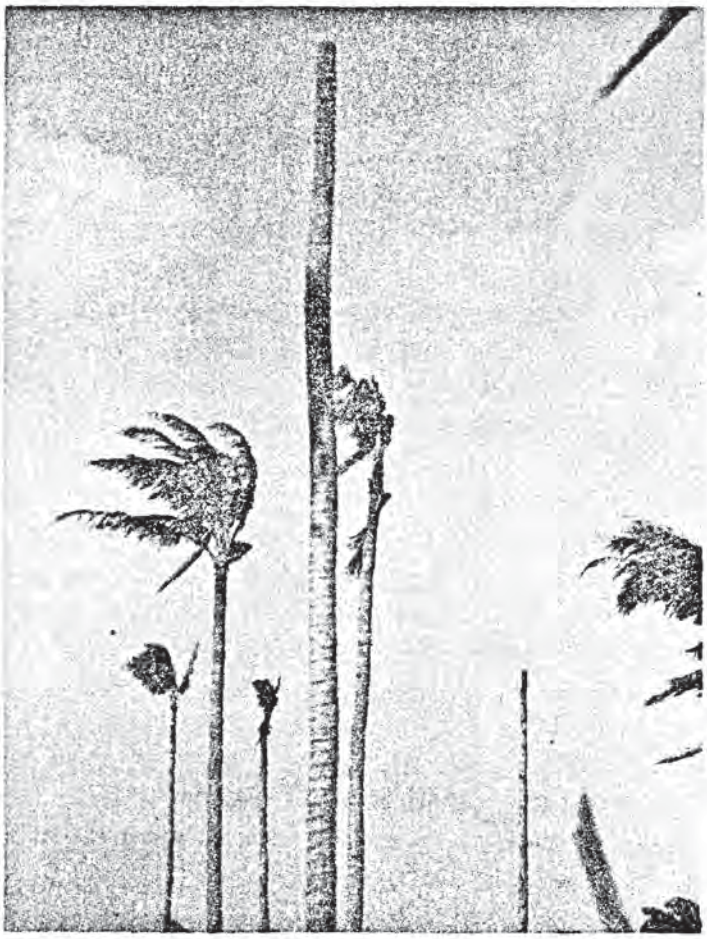


Figure 1. An illustration of the effect of cadang-cadang on the coconut palm. All the palms in the photograph are in a late stage of the disease.

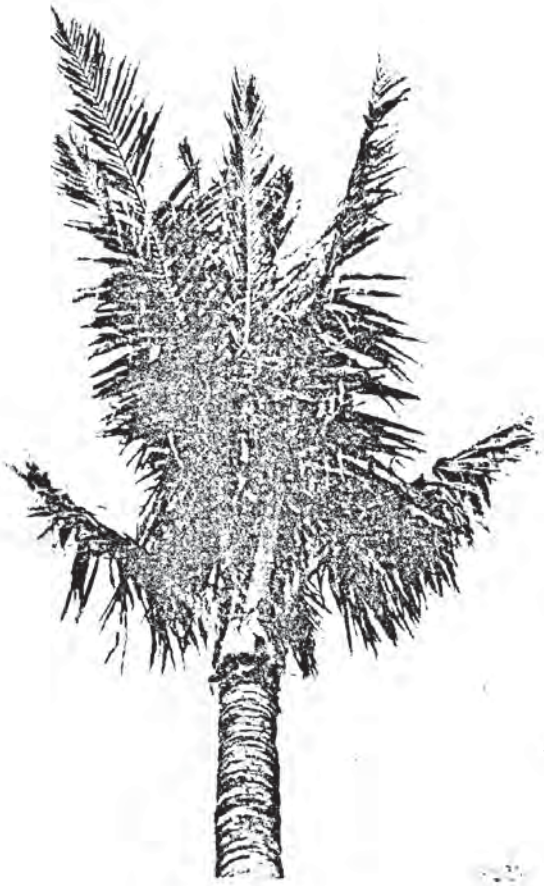


Figure 2. A close-up of a coconut palm in a late stage of cadang-cadang when the lower leaves have dropped and the upper ones have become shortened and brittle, and when the palm has ceased to fruit.

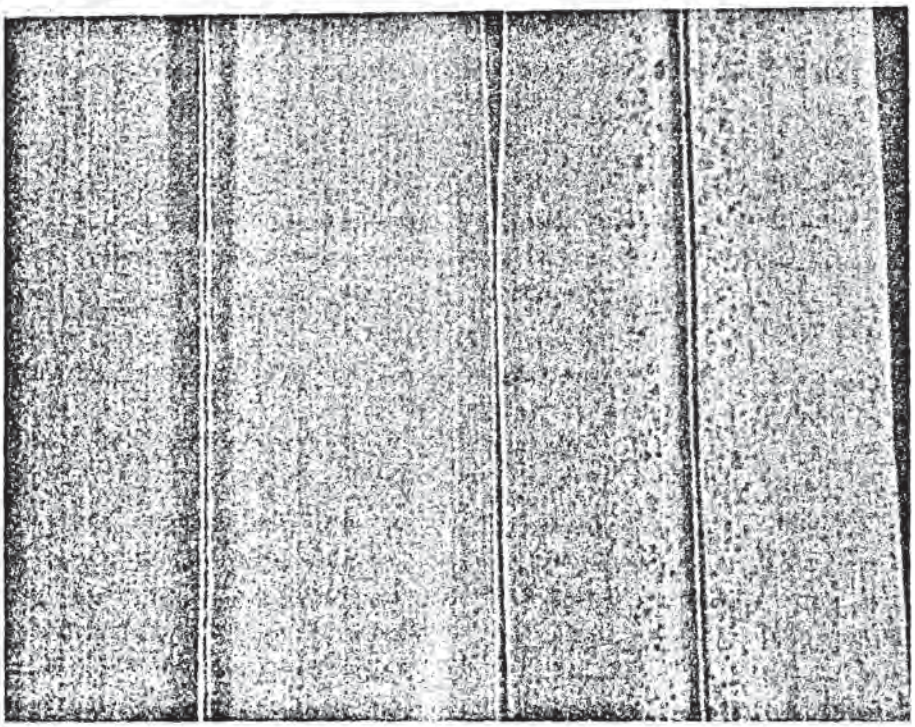


Figure 3. Portion of a pinna from a coconut tree affected by cadang-cadang in comparison with a similar pinna from a healthy tree. The photograph was taken with reflected light to illustrate the olivaceous, or water-soaked appearance of the numerous diagnostic spots that appear on the pinna.

Figure 4. A photograph illustrating the method used to inoculate a young coconut seedling. The stipules have been cut away from the older leaves so as to expose the tender young tissue at the base of the spear leaf, which is then rubbed with a pad containing the inoculum

Figure 5. The plantation in the photograph was practically destroyed by cadang-cadang when it was replanted to coconut. The young coconut replants are continuing to grow well on land on which the disease has killed the previous crop.

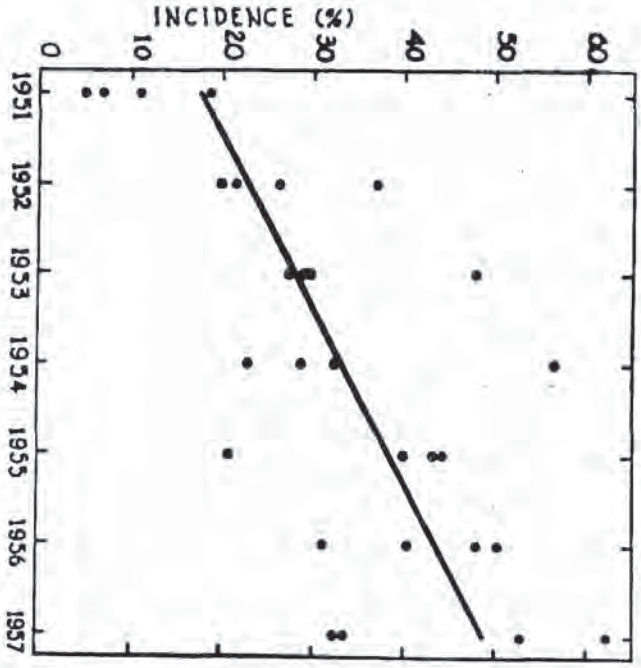
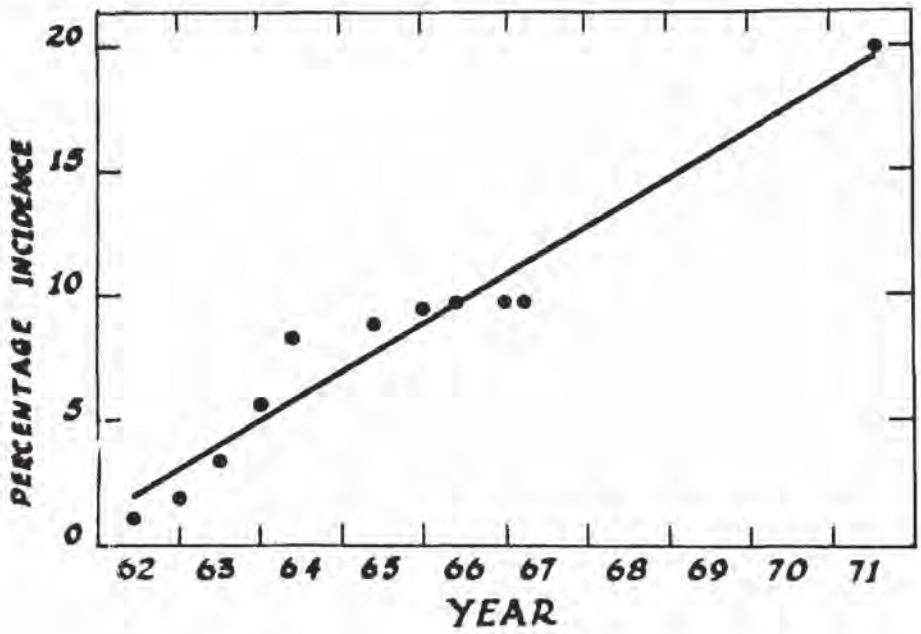
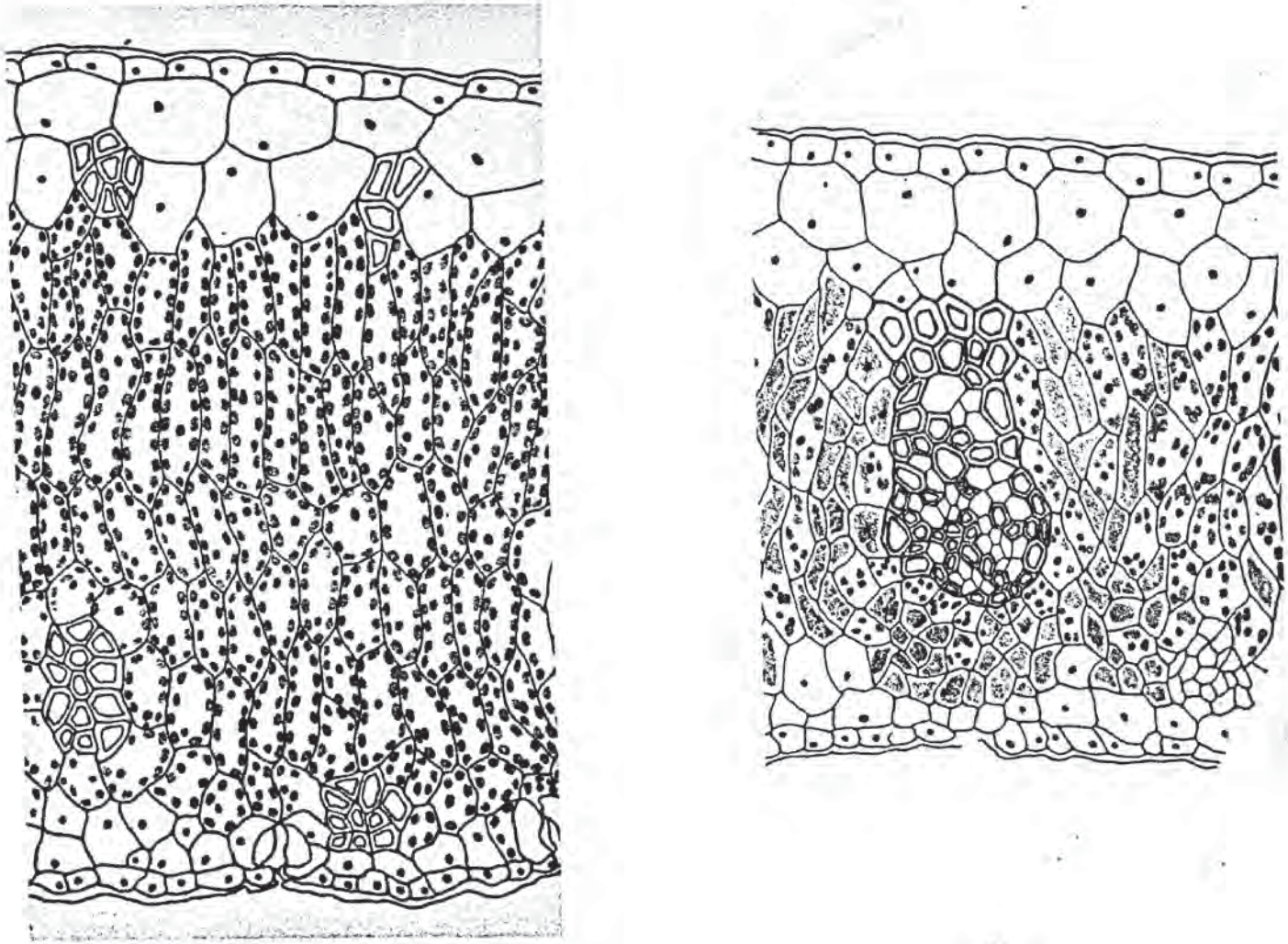


Figure 6. A scatter diagram showing the incidence of cadang-cadang by year from 1951 to 1957 in four provinces of the Bicol region. The incidence appears to have increased in a linear fashion. (Data from Bigornia et al. 1960)

Figure 7. The incidence of cadang-cadang in a weed control plot at Bay, Ligao, from 1962 to 1971. The incidence in this plot seems to have increased in a linear fashion. (Data from Table 1.)





A

B

Figure 8. Camera lucida drawings of cross sections through pinnae of the coconut palm. A) From a healthy palm. B) From a tree affected by cadang-cadang. Cells of the diseased leaf are smaller in size than those of the normal leaf and not so orderly in arrangement; the leaf itself is thinner. The chloroplasts have disintegrated.

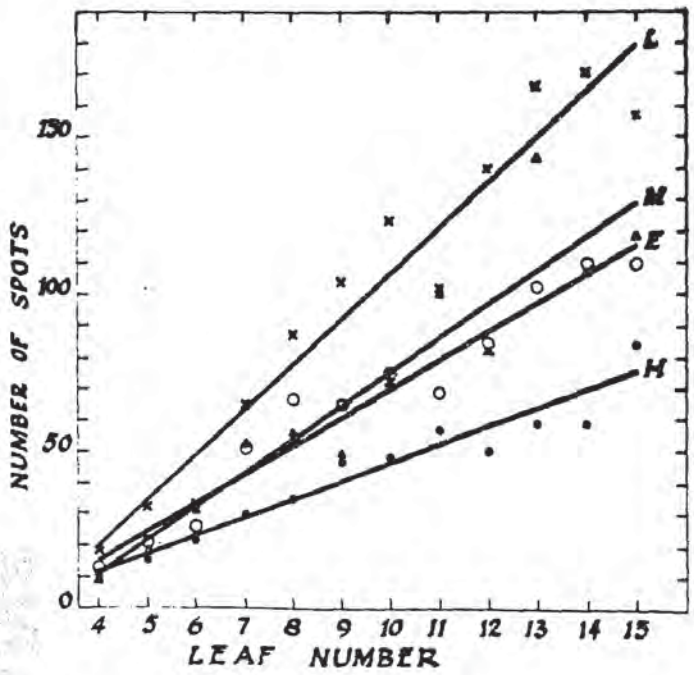


Figure 9. Chart representing the numbers of yellow spots in coconut leaves plotted as a function of leaf position: H) healthy trees; E) early stage of cadang-cadang; M) medium stage; and L) late stage. The numbers increase linearly as the leaf ages.