REPORT ON THE CITRUS RESEARCH WORKS AT CITRUS AND VEGETABLE SEED RESEARCH CENTRE, DHAKA

DECEMBER, 1983

JAPAN INTERNATIONAL COOPERATION AGENCY

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CONTENTS

Actual Condition of Citrus Trees and its Restoration Measures

Isao IWAGAKI

Senior Researcher,
Okitsu Branch, Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries

CONTENTS

Introduction

As a short term citrus expert for the technical cooperation on Citrus and Vegetable Research Project in Bangladesh, I was dispatched to the Citrus and Vegetable Seed Research Centre from March 3 to April 30, 1983.

Because it is the final year of the project according to the Record of Discussions, recommendation of the good rootstocks and selection of promising sion varieties among introduced varieties are included in the duty of the citrus experts. But the research works in the project have not shown enough progress as was expected not only because citrus research takes long period by nature but also because the project has some difficulties.

In those conditions, my activity in the project can be said to have been limited to the restoration of deteriorated citrus trees and initiation of some experiments to solve the technical problems. Those works were conducted with Mr. Kono, citrus expert, with the intention of transfer of the research techniques to the local counterparts. I hope that I could have a general agreement among the persons concerned on my opinion that the research on perenial citrus trees will be possible after piling up the series of fundamental techniques and trials.

I had a chance to visit Regional Agricultural Research Station at Ishurdi and to have a lecture on the occasion of training programme of local citrus growers. Outline of the training programme and the lecture manuscript will be attached as appendices at the end.

I wish to express my sincere gratitude to Dr. H. Sakai, Japanese team leader, Mr. S. Kono, citrus expert, Mr. S. Tasaki, vegetable expert, and Mr. T. Nakagawa, coordinator of the team for their hearted instruction, assistance, and support not only in the research works but also in the private life; to Dr. K. Badruddoza, Director of Bangladesh Agricultural Research Institute, Dr. A. Hossain, and Mr. A. Razzaque for their support to my activities. I am also very much grateful to Mr. A. Abdullah, Mr. A. Khan, Mr. A. Hoque, and other research staff and assistants concerned for their cooperation and friendship during my stay in Bangladesh.

This report was kindly reviewed by Dr. Sakai.

Actual condition of citrus growth

(March-April, 1983)

The result on observation and investigation of citrus conditions during the two-month period after my arrival at the project on March 6 is summarized in the following.

The growth of all citrus varieties was quite poor. Very few varieties such as local Elachi and seedless lemon, and Kawano Natsudaidai retained some previous year's leaves. There were very few previous year's leaves left on wase-satsumas and navel oranges. Most of introduced varieties showed poor tree growth.

Many varieties were already in the stage of flowering on March 6, and flowering was observed until mid March. But, because of poor tree growth and growth irregularity, flowering period of individual variety was not able to be determined.

(1) Flowering and fruiting

The results of observation on seasonal variation of the number of reproductive structures of mandarin varieties are shown in Table 1. The observation was started on February 6 by my request from Japan. All reproductive structures on one scafold branch from each tree were counted. Satsuma mandarin trees which had very few previous year's leaves were selected for the observation.

Reproductive structures which were generated in early February amounted to about 100 per scafold branch at the maximum. The number decreased rapidly, and there were very few fruit left in early April. New leaves which developed simultaneously with flowers were turning to normal deep green in early April without developing their normal size. Most of reproductive structures dropped in their bud stage and only 10 percent of them flowered.

Seasonal variations in the number of reproductive structures of citrus varieties are shown in Table 2. This observation was started after my arrival at the project selecting 5-year-old trees of 2 local

Table 1. Seasonal variation of number of reproductive structures of 4 satsuma mandarin varieties

Varieties	Reproductive structure	February				March		April
		6	13	20	27	10	17	4
Okitsu-wase	Bud	9.4	4.2	72.2	82.4	60.0	0.6	0
	Flower	2.8	2.6	0	9.8	5.4	0.8	0
	Fruit	2.4	7.6	8.0	13.0	10.2	3.6	0
	Total	14.6	14.4	80.2	105.2	75.6	5.0	0
Miho-wase	Bud	14.4	16.2	19.0	51.6	5.0	0.2	0
	Flower	5.6	5.2	5.8	14.2	0.6	0	0
	Fruit	4.8	2.0	4.8	6.4	7.8	0.2	0
	Total	24.8	23.4	29.6	72.2	13.4	0.4	0
Miyagawa-wase	Bud	0	0	10.4	12.8	7.8	1.0	0
	Flower	0	0	0	4.6	0.2	0.4	0
	Fruit	0	0	0	1.0	2.6	0.8	0.3
	Total	0	0	10.4	18.4	10.6	2.2	0.3
Tanikawa	Bud	0	0	27.0	36.8	15.4	0.4	0
	Flower	0	0	0	0	2.8	0.6	0
	Fruit	0	0	0	0	3.6	2.4	0.6
	Total	0	0	27.0	36.8	21.8	3.4	0.6

Field No. 2, No. 5 Plot
Scafold branch basis
Average of 5 trees.

Table 2. Seasonal variation of number of reproductive structures of 5 citrus varieties

Varieties	March			April		
	8	15	22	3	12	24
Elachi-lebu	220.5	106.0	31.5	5.5	5.0	3.5
Seedless lemon	143.0	52.0	24.0	87.0	99.5	29.0
Hyuganatsu	376.0	363.5	147.0	34.5	17.0	14.5
Kawano Natsu	269.0	66.0	26.0	16.5	3.5	0
Miho-wase	426.5	40.5	10.0	1.0	0	0

Field No. 2, No.3 and 5 plots
Whole trees basis
Average of 2 trees.

and 3 introduced varieties which had retained comparatively many previous year's leaves. All reproductive structures on those trees were counted.

Although there were many buds and flowers developed on introduced varieties, the number of them decreased rapidly towards early April. There were relatively few buds on local varieties. Elach-lebu buds showed the same decreasing trend as introduced varieties. Seedless lemon formed secondary flower buds after mid March. Fruit bearing percentage of those buds and flowers were not determined until the end of April but it was estimated that there would be almost no fruit except seedless lemon.

(2) Tree growth

Tree growth observation and measurement were done on April 5 at No. 5 plot where the trees were planted in 1979. The results on 22 varieties which more than 3 original planting were affirmed are shown in Table 3.

Only 4 varieties viz. local Elach-lebu and seedless lemon, and introduced Beni-hassaku and Kawano Natsudaidai showed better tree growth. The total number of vigorous and intermediate growth trees was beyond 50 percent against the number of original planting in those 4 varieties. Two local varieties were large in tree volume

Table 3. Tree growth observation on 5th, April, 1983

Varieties[1]	Rootstock	Original tree No.[2]	Vigorous or Intermediate tree No.[3]			Percentage of V and I trees	Tree Volume[4]
			V	I	Total		
Elachi-lebu	Layering	9	4	3	7	77.8 %	3.90 m^3
Kagzi-lebu	L	7	0	0	0	0	-
Seedless lemon	L	18	5	4	9	50.0	4.71
Kinnow	Pomelo	9	0	0	0	0	-
Dancy	P	8	0	2	2	25.0	0.38
Jaffa	P	8	0	0	0	0	-
Feutrell's early	P	8	0	0	0	0	-
Mosambi	P	12	0	2	2	16.7	0.35
Suckery	P	3	0	0	0	0	-
Ruby red	P	3	0	0	0	0	-
Pineapple	P	9	0	0	0	0	-
Hyuganatsu	Trifoliate	18	0	0	0	0	-
Yoshida navel	T	8	0	0	0	0	-
Beni-hassaku	T	9	3	2	5	55.6	1.15
Kawano Natsu	T	9	1	6	7	77.8	1.72
Kawachi-bankan	T	4	0	0	0	0	-
Lisbon	T	9	0	2	2	22.2	1.97
Ureka	T	9	0	0	0	0	-
Villafranca	T	9	0	4	4	44.4	0.98
Miyagawa-wase	T	9	0	2	2	22.2	0.65
Okitsu-wase	T	18	0	5	5	27.8	1.68
Miho-wase	T	27	0	10	10	37.0	1.40

Field No. 2, No. 5 plot

1) In the order of field layout

2) Planted in July, 1979

3) Tree condition was classified into vigorous, Intermediate and weak

4) Tree volume = North-South diam. x East-West diam. x height x 0.7
Average of Vigorous and Intermediate trees.

with high and wide tree dimensions but low in leaf density. Two introduced varieties were high but thin in tree spread and very low in leaf density. As a whole, tree growth of citrus varieties was very poor, so research work on them was considered to be impossible at this stage except on local varieties.

(3) Analysis of poor tree growth

Some problems related to poor tree growth are discussed in the following. Insect pests observed during my duty period were citrus aphids, lemon butterflies, scale insects, leaf miner, etc. Appropriate control measures are necessary for those insect pests though it was thought that insect pests were not the major factors of poor tree growth.

Twig dieback, gummosis, canker and sooty mold are major diseases in the project. The former two diseases which are very difficult to be controlled were judged to have much responsibility to the poor tree growth. Twig dieback was observed from the beginning of March. New growth died out immediately after sprouting and the death was spreaded to the previous year branches. Flower buds and flowers dried up and remained on dead twigs. New leaves also dropped in early stage without reaching normal size. The death of branches extended in some cases to older branches and the extension had not ceased at the end of April. Wase satsumas which had retained very few old leaves suffered from dieback most seriously. When tree growth deteriorated due to twig dieback of older branches, gummosis occurred on scafold branches and trunks in a high rate.

Moisture stress at the terminal of branch may be the primary cause of twig dieback. Heavy clay soil has hazardous problems of excessive water and oxygen deficiency in the root zone in rainy season. It may also cause the wet injury of root and restrict proper development of root system. High temperature and low soil humidity in the succeeding dry season further the water stress. Intensive soil improvement and proper control of soil moisture should be employed to solve these problems.

There is an opinion among local researchers that the poor tree growth of current dry season is due to the crop load of last year but this opinion should be denied because the number of harvested fruits was about 10 per tree at the most. Before my arrival at the project, Dr. Sakai and Mr. Tasaki had started to improve the drainage and water intake of a planting hole making a small ditch connecting each planting hole with the furrow. Physical property of soil and soil moisture condition of the field will be discussed later. Chemical property of soil and problems on mineral deficiency and greening disease will also be discussed later.

(4) Shade trees

Ipil-ipil as shade tree in the field has been removed by digging out or by applying glyphosate. Ipil-ipil trees were planted at 20 cm distance from the citrus trunk in some cases and large root of them intruded under citrus trees. All ipil-ipil trees should be discarded except when they are of use as wind breaks. Shade trees may have some reasons of existence in Bangladesh but our conviction is that the demerit of it may outpace the merit.

We commenced shade trial on March 17 to investigate the effect of shade on citrus growth (Table 4). Potted 3-year-old trees of 6 varieties were used. There are 2 plots viz. control (under the direct solar ray) and shade (about 80 percent of solar ray was cut with black cheese-cloth) in 3 replications. Green color of shaded citrus tree leaves was deepened on 21 days after treatment and the color difference between control and shaded tree leaves was enlarged thereafter. Dark green color of shaded tree leaves is a proof of shading effect. The trial will be continued to investigate the tree growth.

Table 4. Effect of the shade on the grade of green of potted citrus variety leaves

Varieties	April 7		April 26	
	Sun	Shade	Sun	Shade
Elachi-lebu	4.7	6.7	4.1	6.1
Seminole	2.0	3.3	2.7	4.9
Tachibana	3.0	4.7	4.4	6.9
Beni-hassaku	2.6	3.7	3.7	5.1
Matsuyama	2.2	3.3	3.1	5.4
Tanikawa	3.3	3.6	4.1	5.6

Leaf color was rated using the standard (1: light green to 9: deep green) of Nagasaki Fruit Tree Experiment station. Three spring cycle leaves of current yer per plant were measured and averaged. Average of 3 reprications.

Soil and root system observation

Joydebpur is on the Mohdhupur plateau in the center of the country. It is flat in topography and has poor drainage problem. The soil is called Kalmachiata soil series and soil texture is heavy clay.

Physical property of the soil of citrus field in the project has not been reported yet. We conducted soil profile observation and measurement of actual volume and hardness of the soil.

Selecting seedless lemon, Miho-wase and Adajamir trees of medium vigor, soil profiles were made at 40 cm east side of the trunk of each tree in Field No. 2. Surface soil was somewhat loose by plowing but subsoil was very compact. There was very few difference in soil compactness from 5 cm below the surface to deeper subsoil. Soil color was light gray to yellow-brown at the surface and it changed to red-brown with vertical mottling of iron oxide below 20 cm.

(1) Three phase of soil

The results on three phase of soil by soil actual volumenometer and soil hardness by soil hardness tester are shown in Fig. 1. The measurement was conducted on March 14 and 15.

Solid phase was about 50 percent at the soil surface and 50 to 60 percent below 10 cm depth. The results demonstrated high compactness of the soil.

Liquid phase was 21 to 26 percent at the soil surface, 27 to 33 percent at about 10 cm depth and it went lower again at the deeper soil layer. It was the latter half of dry season when the measurement was conducted and the precipitation before measurement was 10.6 mm in January, 23.4 mm in February, and no rain in March. The last irrigation in the field was 3 to 11 days before measurement. Under these conditions, low water content of surface soil and subsoil, and higher water content of 10 cm depth by irrigated water were revealed by the measurement.

Air phase percentage was 26 to 37 percent at the soil surface and it went lower than 20 percent below 10 cm depth. In some cases,

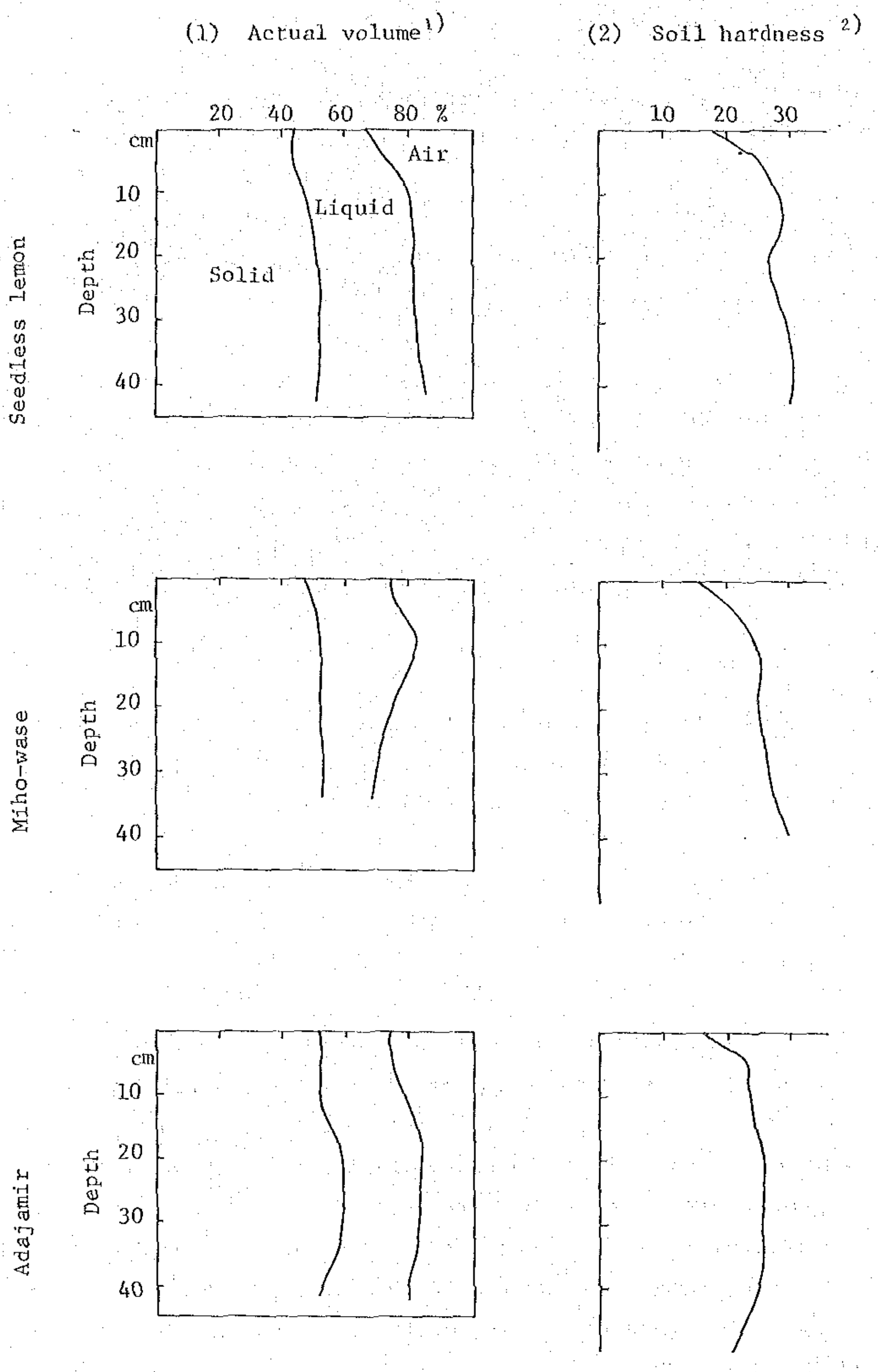


Fig. 1 Actual volume and hardness of soil at 3 spots in Field No. 2

1) Soil Actual Volumenometer, Model 100

2) Soil Hardness Tester, Yamanaka Type

in proportion to the decrease of water content in the subsoil, percentage of air phase increased.

(2) Soil hardness

Soil hardness was about 15 at the soil surface and higher than 20 below 5 cm. The value was 25 to 30 in the subsoil.

Above mentioned physical property of the soil is considered to be the factor to limit the growth of citrus root.

(3) Root system

Root system of three citrus varieties was observed. Although the density of root was low, medium and large root were distributed mostly in the 5-20 cm soil layer. Root distributing layer coincided with the high water content layer. Very few root was found in other soil depth. Horizontal distribution of root reached 100-150 cm from the trunk in 5 cm depth. As a whole, fine root distribution was quite limitted.

Regarding the Adazamir root system, it was found that horizontal distribution was limitted and some medium roots were found in the 40 cm depth. Adazamir was regarded to have deep-root characteristic.

(4) Soil improvement

Through the investigation of physical property of the soil and observation of root system we found that the soil of Field No. 2 was not congenial to citrus growth. Recognizing the need for intensive soil improvement, we employed two methods viz. complete improvement and partial improvement of soil condition in the root zone. Schematic diagram of position of soil improvement is shown in Fig. 2. Improvement of physical and chemical property of the soil was conducted by replacing the soil of root zone by equal mixture of cowdung, sand and soil. Partial improvement was employed as an easier method with an aim to facilitate the water movement from planting hole to furrow.

Concerning to the soil moisture control during the dry period, regular irrigation was recommended.

(5) Chemical property of soil

Analytical data of soil has been reported by Sakai. The soil of Field No. 2 is low in total nitrogen, NO_3-nitrogen and exchangeable potassium. The soil is very low in available phosphate, sulphur, and zinc.

(6) Leaf analysis

Analytical results of citrus leaf by Sakai are shown in Table 5. Nitrogen level is low in most cases and sulphur is in deficient level. Deficiency of those element may result in deterioration of green in the leaves. Sakai has also reported the possibility of manganese deficiency and zinc deficiency and suggested the wet injury of root system because of high iron content in leaves.

Although we also observed the symptoms of maganese deficiency and zinc deficiency, we could not specify the deficiencies because the tree growth was generally poor and those deficiency symptoms were not easily discriminated from the symptom of greening desease. Bangladesh citrus is considered to be contaminated with greening disease which is widely distributed in tropical South east Asia. Delay in the study of greening is thought to be partly responsible for lack of proper diagnosis and formulation of proper cultural managements.

Urea, triple superphosphate and muriate of potash had been used for citrus fertilization in the project. Annual fertilization of those non-sulphate fertilizers may further the sulphur deficiency because the soil of citrus field in the project is low in sulphur content. Fertilizing programme was revised adopting the fertilizers containing sulphur viz. ammonium sulphate, superphosphate and potassium sulphate.

Fertilizer trial was started to determine growth response to fertilizers containing sulphur in contrast to the non-sulphate fertilizers. Foliar application of zinc sulphate was also started.

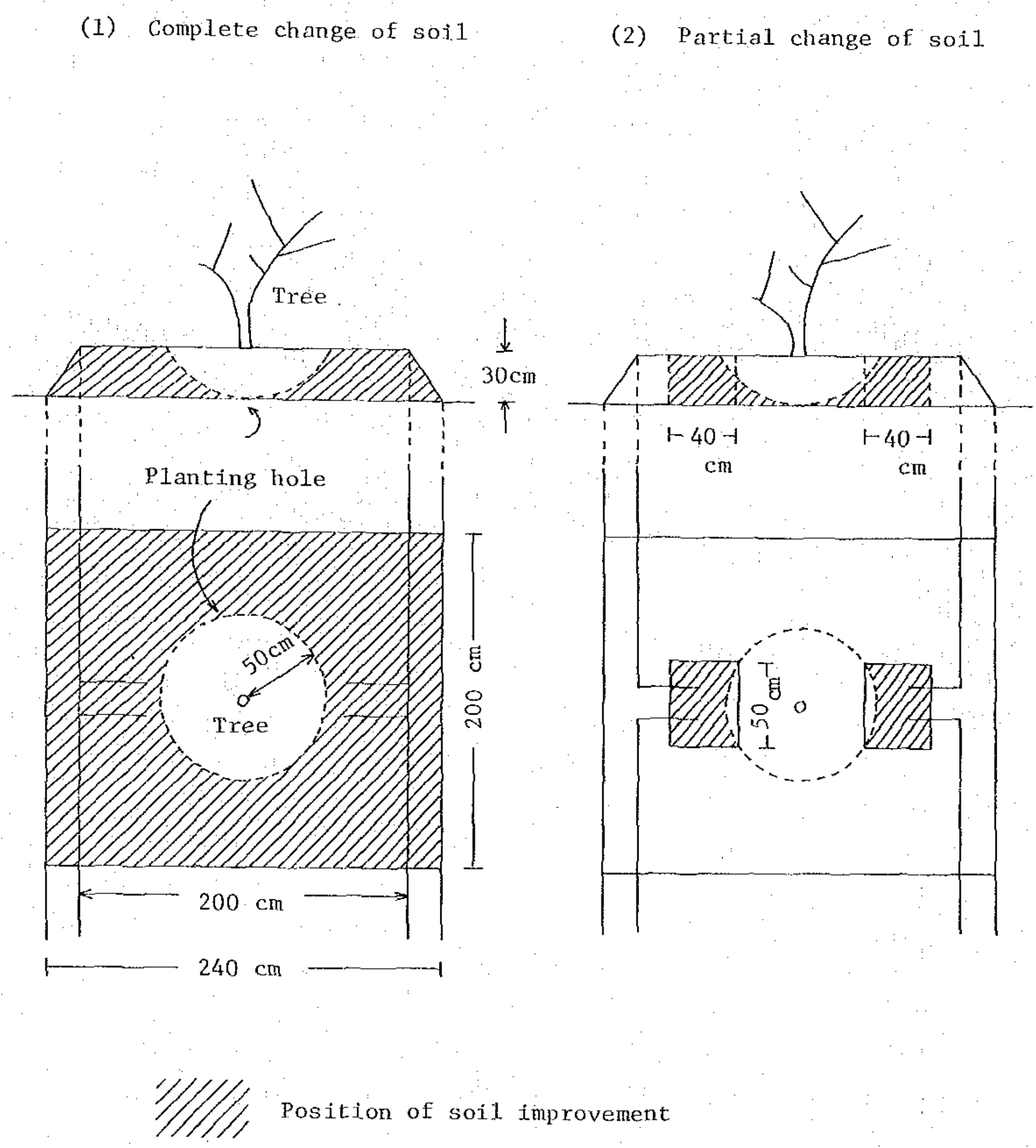


Fig. 2. Schematic diagram of position of soil improvement

Table 5. Analytical results of citrus leaf

Field	Varieties	N	S	N/S	Fe	Mn	Zn	Cu	Mn/Fe
		%	%		ppm	ppm	ppm	ppm	
Well drained field	Okitsu-wase	2.75	0.172	16.0	137	29.4	34.2	6.90	0.215
	Kobayashi-mikan	1.82	0.114	16.0	199	27.6	45.8	9.66	0.139
	Sambokan	2.30	0.125	18.4	170	12.1	69.4	-	0.071
Poorly drained field	Pummelo	1.90	0.131	14.5	375	43.2	33.3	6.90	0.115
	Lemon	2.35	0.160	14.7	275	58.7	66.7	6.55	0.213

Field No. 2. Oct. 24, 1982

Analyzed by Sakai.

Summary and recommendation

Growth of all the citrus varieties is quite poor. Very few varieties such as local Elachi and seedless lemon, and Kawano Natsudaidai retained some previous year's leaves. There were very few previous year's leaves left on wase-satsumas and navel oranges. Most of introduced varieties showed poor tree growth.

Survey of the seasonal variation in the number of reproductive structures of some citrus varieties revealed that a few fruit remained on the tree except local varieties.

Though we made intensive observation and measurement, it is difficult to recommend suitable varieties in this country because our information was just collected under a certain fixed condition such as; rootstock, cultural practice, soil, climate, age of plant etc. We dare to list Hassakus, Natsudaidais and Tankans among introduced varieties as probable candidates for this country.

Scale insects, twig dieback and gummosis were observered to be the major pests and diseases. Probable cause of twig dieback and gummosis is related with soil and water condition. We conducted soil profile observation of the experimental fields and found that the physical character of the soil was not congenial to the expansion of root system. Considering the urgent need for soil improvement, we employed two methods viz. complete improvement and partial improvement of soil condition in the root zone. It is hard to believe that no step had been taken to improve the soil condition until the end of last growing season. Regular irrigation during the dry period is recommended.

Analytical data of soil has been reported by Sakai. The soil is low in nitrogen, phosphate, exchangeable potassium and sulfur. We have observed some manganese and zinc deficiencies as Sakai has reported from his analytical findings. But those deficiency symptoms are not easily discriminated from the symptom of greening disease. Delay in the study of greening is thought to be partly responsible for lack of proper diagnosis and formulation of proper management.

Fertilizer trial to determine the plant responses to fertilizers containing sulphur in contrast to the non-sulphate fertilizers which are popular in this country was started. Fertilizing programme was revised adopting the fertilizers containing sulphur. Foliar application of zinc sulphate was also started.

Some trees were planted too deep in the soil to make healthy growth. It was suggested to move the trees up. Because of the weak growth of trees, training and pruning practices were thought to be ineffective, but pruning procedures were demonstrated.

Some rootstock trials are under way, but to recommend good rootstock in this country is next to impossible. The potential rootstocks for this country may be local pomelo, Trifoliate orange, Yuzu, sour orange, rough lemon etc.

We abandoned shade trees except those which are of use as windbreaks. Our trial on the effect of shade on tree growth will support our conviction. The recommendations for removing shade trees will be appreciated when proper care of the citrus trees as has been mentioned here and/or in other places is fulfilled.

Appendix - 1

Training programme of local citrus growers

Regional Agricultural Research Station

Bangladesh Agricultural Research Institute

Ishurdi, Pabuna

April 18, 1983

10.30	Inauguration by Dr. K. Badruddoza, Director, BARI	
11.30	Tea break	
12.00	Scope and importance of citrus cultivation in relation to Bangladesh	Mr. A. Razzaque
12.30	Vegetative propagation of citrus	Mr. A. Abdullah
13.30	Lunch and Prayer	
14.30	Propagation of citrus (Theoretical and practical)	Mr. I. Iwagaki
17.30	Tea break and close	

April 19, 1983

9.00	Planting and other intercultural operations	Mr. A. Hoque
10.30	Cultivation of citrus in Japan	Mr. K. Kono
11.00	Tea break	
11.30	Diseases of citrus plants and their control	Mr. A. Khan
12.00	Insect-pest and their control	Mr. A. Mannan
13.00	General discussion	
13.30	Lunch and Prayer	
15.00	Pruning practice in farmer's orchard	Mr. I. Iwagaki
18.00	Tea break and close	

Appendix - 2

PROPAGATION OF CITRUS

Isao Iwagaki

There are many ways of propagating citrus trees. Among them, seedling, air layering and employment of rootstocks are believed to be practical for the production of nursery trees in this country. At the first place, it may be worth-while to discuss the merits and demerits of those methods.

(1) Seedling:

Though it is an easy method, it has a lot of problems for growing seedling up to fruit bearing stage. In the case of monoembryonic varieties such as pomelo, you can not get the same kind of trees to mother plant. Each seedling shows different character inherited from father plant. Even in the case of polyembryonic varieties some variation may exist among seedlings. In many cases, root systems of seedlings of cultivated varieties are less tolerant to adverse soil and water conditions. Seedling root may have unfavourable effect on production and fruit quality. Seedling is slow to reach the fruit bearing stage.

(2) Air layering:

Air layering is widely adapted for lemon and lime propagation in this country. You can get uniform nurseries which have the same quality to mother plant by layering. But the problem of defective root system may often arise in such a way as seedling has. Layering is not practical for mass propagation.

(3) Rootstocks:

Most commercially-grown citrus trees in the world are grafted to seedling rootstocks of a variety grown for that purpose. Rootstocks are chosen on the basis of scion compatibility, adaptability to soil and water conditions, ultimate trees size, favorable effect on production, fruit size and quality, and disease resistance. Every citrus variety under cultivation should have the suitable rootstock. But rootstock trials take so long period that even in advanced countries the trials are not yet completed.

Pomelo has been only one rootstock variety of citrus in this country. The seedling of pomelo shows very rapid growth, and reaches the optimum size for badding very quickly. But the root system consists of only taproot having scantly lateral roots. Accordingly the transplanting is very difficult in the nursery bed as well as in the orchard. Furthermore, compatibility between pomelo and other group of citrus (orange, mandarin etc.) seems to be not so good as revealed in the previous trials and observations. As pomelo seed is monoenbryony as mentioned above, efforts to select uniform seedlings should be made.

Trifoliate orange is most extensively used as rootstock in Japan especially for mandarins. The seedling of trifoliate orange shows slow growth. So, more than one year is needed to perform budding operation on them. The root system of trifoliate orange consists of only lateral root having no taproot, that is to say, the citrus varieties of trifoliate orange are tolerant against transplanting. Trifoliate orange is a good rootstock for some of loose skin mandarins, especially good for satsumas.

Since the trial to find the best rootstock is a long term work, it should be started immediately. Sour orange, rough lemon, Rangpur lime, Adazamir and Khatti are some of candidates.

Seed bed preparation

Sandy soil needs more frequent watering and fertilization. Clay soils often remain wet and prevent development of fibrous root. It is usually more satisfactory to use medium textured soil, even if it has to be prepared.

As the seedlings emerge they probably need some sort of shelter to reduce the sun light intensity.

Selecting bud wood

Use buds from the best producing, true-to-type, disease free trees for propagation. Where possible, bud each-block of nursery trees from a single parent source. This helps to insure more uniform tree behavior in the orchard.

Grafting technique

(1) Budding:

Use the shield or "T" bud method as illustrated in the figures. Properly done, it gives excellent results, with a minimum effort. A good sharp knife must be used to slice buds from the budsticks. Large bud for insertion into pencil sized and larger stocks should be cut up to 2 to 3 cm long and deep enough to include a sliver of wood. Smaller buds may not include wood.

In wrapping buds, start below bud and work up to avoid moving the bud out of place. Tie buds firmly but not so tightly as to impede growth.

(2) Grafting:

Grafting can be used in a season when the sap movement is not active and the rootstock bark is not easily pealed for budding. Thorny rootstock varieties can be easily grafted when you cut the rootstock top off. Use small thin vinyl bags to protect the scion stick to wilt.

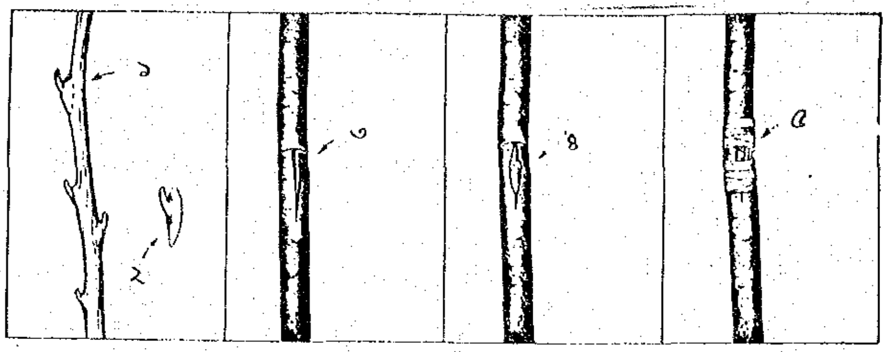
Taking care of nurseries

Usually you may remove the wrap after 2 to 3 weeks of good growing condition. Cut off the top 1 cm above the bud just after new growth starts.

To develop straight trunk, citrus bud shoots usually require some support. The tie must be tight enough to prevent the budlings from whipping due to strong winds, but the ties must not pinch the shoot. Remove all suckers by rubbing off before they grow so big that they have to be cut with shears. It is recommended to encourage the bud shoots to grow straight up as high as they will in one season. This procedure eliminates a certain amount of suckering and usually produces trees of larger diameter. The trees must be cut back to 30 to 40 cm above the ground at the time of transplanting. It has good effect on balancing the growth of top and root.

(১)

চোখ কলম করণের বিভিন্ন ধাপ



(১) চোখটি কেটে নিন, (২) চোখটি এরকম দেখাবে, (৩) "T" এর মত করে চারাটির গায়ে কাটতে হবে, (৪) চোখটি কাটা অংশে ঢুকিয়ে দিন, (৫) প্লাস্টিকের ফিতা দিয়ে বেঁধে দিন।

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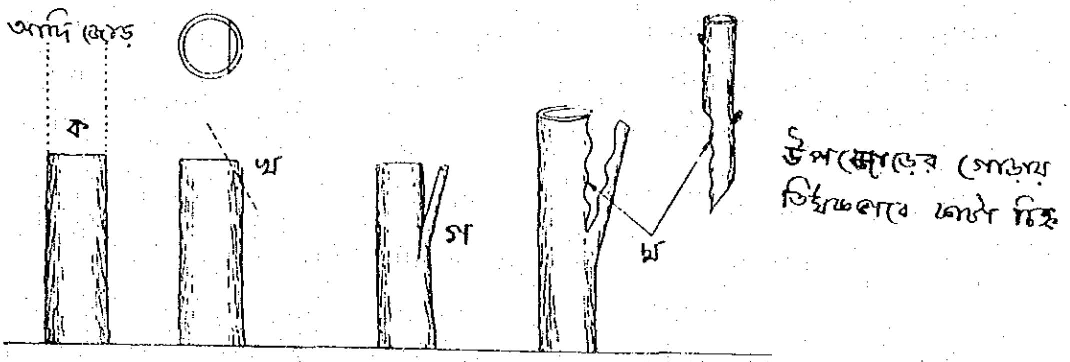
জোড় কলম করণের বিভিন্ন ধাপ



(ক) মাথা কাটা চারা, (খ) পার্শ্বের এক অংশ কাটা, (গ) লম্বালম্বি ভাবে কাটা, (ঘ) অসমান ভাবে কাটা উচিৎ নয়।

বি: দ্র: স্টক ও সায়ন এর ক্যাম্বিয়াম মিলে যেতে হবে।

Appendix - 3

Monthly weather report

Meteorological Services Section

Bangladesh Agricultural Research Institute

Joydebpur, Dhaka

Month	1982			1983		
	Temperature(°C)		Precipitation(mm)	Temperature(°C)		Precipitation(mm)
	Max	Min		Max	Min	
1	27.5	14.5	0	25.8	14.9	11
2	27.9	16.2	7	27.4	14.9	23
3	31.7	21.4	160	32.7	22.4	206
4	34.0	23.9	222	33.8	23.4	167
5	35.6	26.1	194	34.2	25.7	335
6	32.2	27.0	516			
7	33.2	27.5	153			
8	31.9	27.2	275			
9	33.4	26.8	151			
10	33.3	24.1	31			
11	28.4	18.3	28			
12	27.0	14.8	0			
Avg.	31.3	22.3	1737			

Appendix - 4

Estimate of citrus acreage and production in Bangladesh

		1972-73	73-74	74-75	75-76	76-77	77-78	78-79	79-80	80-81
Pomelo	acres	1,905	2,016	2,140	2,185	2,305	2,450	2,565	2,625	2,735
	tons	3,317	3,269	3,374	3,495	3,491	3,709	4,015	4,039	4,190
Mandarin	acres	2,071	2,260	2,300	2,295	2,285	1,890	1,930	1,928	1,925
	tons	3,548	3,617	3,522	3,523	3,500	2,360	2,384	2,393	2,395
Lemon and lime	acres	3,622	3,721	3,740	3,925	3,620	3,585	3,750	3,900	4,020
	tons	3,960	3,781	3,532	3,822	3,395	3,272	3,474	3,395	3,653
Total	acress	7,598	7,997	8,180	8,405	8,210	7,925	8,245	8,453	8,680
	tons	10,825	10,667	10,428	10,840	10,386	9,341	9,873	9,827	10,238

Source: 1982 Agricultural yearbook of Bangladesh, Bangladesh Bureau of Statistics

Plates

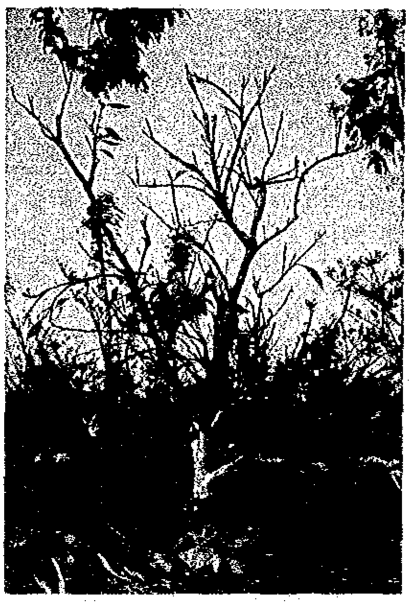
A. Outlines of Citrus and Vegetable Research Project and tree conditions.

Field No. 2, No. 2 plot.
Introduced varieties. March 8.

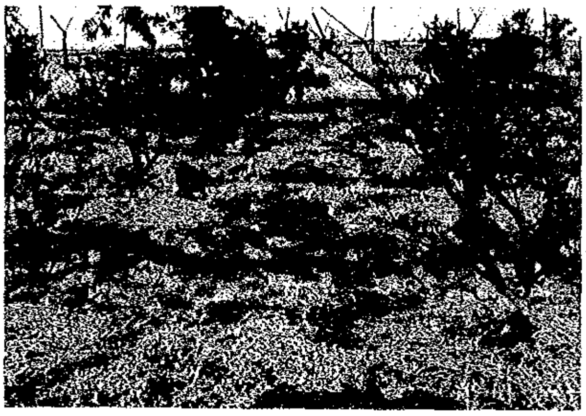
Intertillage, weeding. March 15.

Irrigation. March 8.

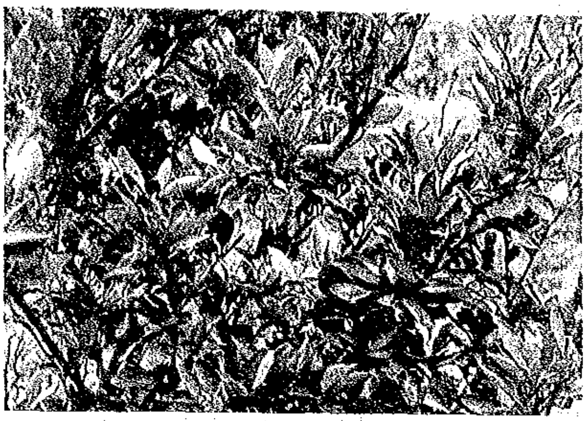
Twig dieback. Wase satsuma.
March 10.



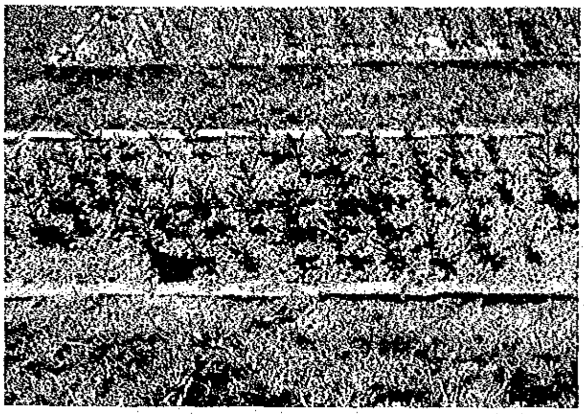
Dying tree by twig dieback.
Wase satsuma. March 13.



Tree condition in No. 5 plot.
Wase satsumas. April 24.

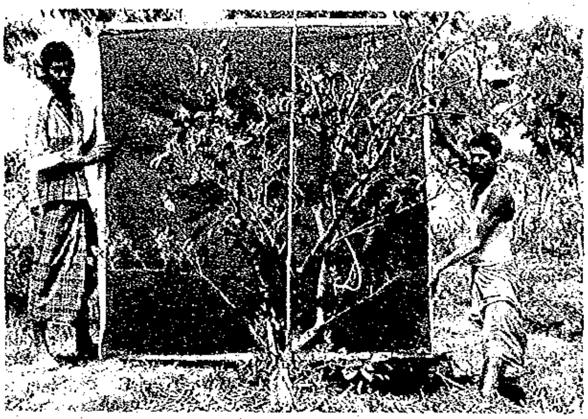


Wase satsuma tree in better condition. April 24.

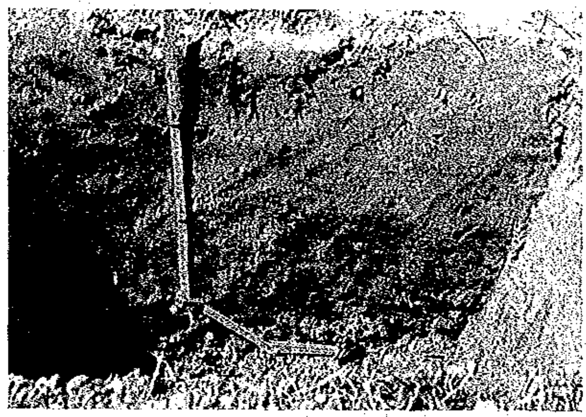


Seedlings of local pomelo for rootstock. March 7.

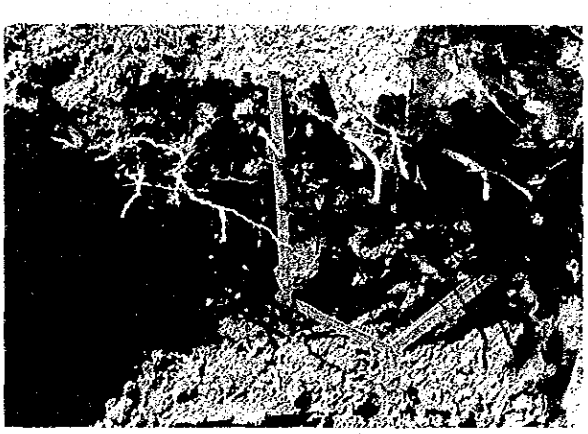
B. Research activities.



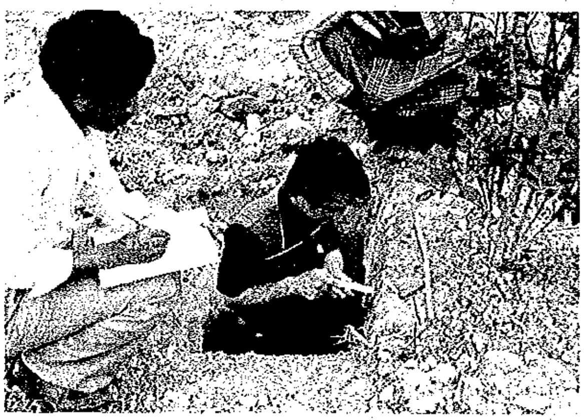
Hyuganatsu. March 8.



Soil profile in No. 5 plot. March 14.

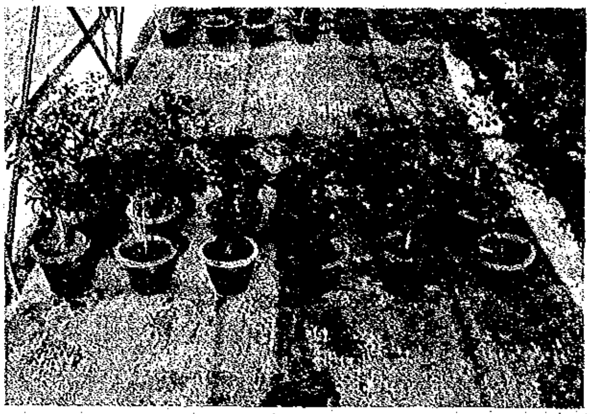


Root system of seedless lemon, same to picture No. 10. March 7.



Soil hardness measurement. March 15.

Soil improvement. April 12.

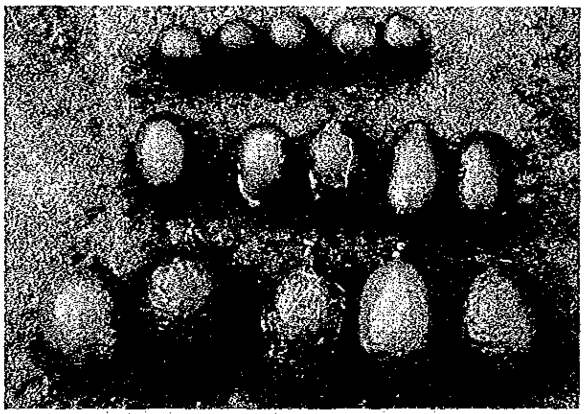


Shade trial. March 17.

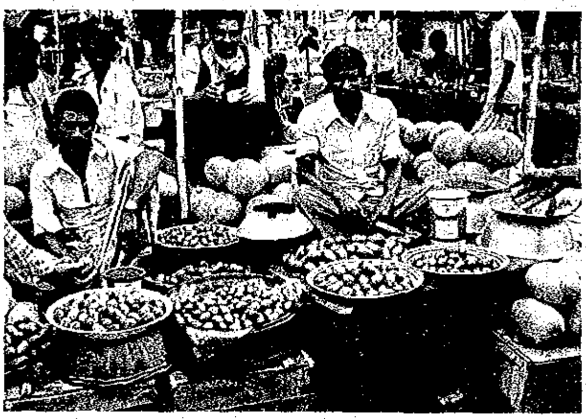
Foliar application of zinc sulphate. March 24.

C. Others

Seedless lemon orchard at Cittagong University. March 30.



Kagzi-lebu, seedless lemon and Elachi-lebu from top to bottom at Ishurdi. April 18.



Local citrus fruit at Joydebpur. April 11.

Diagnostic Aspects of Citrus Diseases in Bangladesh, Emphasizing Overwhelming Prevalence of Greening Disease

Meisaku KOIZUMI

Senior Research Plant Pathologist
Kuchinotsu Branch, Fruit Tree Research Station,
Ministry of Agriculture, Forestry and Fisheries,
Kuchinotsu, Nagasaki 859-25, Japan

INTRODUCTION

The author was dispatched to the Citrus and Vegetable Seed Research Center (CVSRC), Bangladesh Agricultural Research Institute (BARI), Joydebpur, Dhaka under the auspices of the Japan International Cooperation Agency (JICA) from June 4 to July 31, 1983, to investigate citrus diseases and to provide some suggestions to solve the problem. Citrus production in Bangladesh is still undeveloped. Technical cooperation for development of citrus production was started by JICA in 1978; however, large numbers of citrus trees, including local cultivars and introduced varieties, were not vigorously grown. Except for lemon, sour lime or pummelo, the trees have borne few fruit. Poor or low fruit production has been attributed to inadequate conditions such as heavy clay soil, high soil-moisture during rainy season, high temperature, nutritional deficiency and inappropriate rootstock. However, the primary factor was not determined. Based on the investigation in Bangladesh and electronmicroscopy of samples brought to Japan, the main causal factor is probably greening disease, which is widespread in southern parts of Asia including India. In addition, occurrence of another serious disease, gumming, is closely related to the infection with greening disease.

During my stay in Bangladesh, field surveys were carried out in the citrus fields of CVSRC-Joydebpur maincenter, CVSRC-Ishurdi subcenter and Agricultural Research Subsation (ARS), Jaintiapur, in cooperation with Mr. S. Kono, Japanese citrus expert, Mr. M. Bari, SSO, plant pathologist, Mr. M. A. Hoque, SSO, citrus agronomist, ARS-Jaintiapur, and Mr. H. Nagai, Japanese junior expert for citrus. Especially, Mr. Kono coworked fully on the research in the country. Dr. H. Sakai, the Japanese Team leader of CVSRC and Messrs. S. Tasaki and T. Nakagawa, collegues of the project, provided helpful suggestions. Mr. A. Razzaque, director in charge of CVSRC, and Mr. A. M. Abdullah, PSO, provided research-work environment. Dr. E. L. Civerolo, Horticultural Science Inst., Beltsville Agr. Res. Center, USDA, and Mr. C.N. Roistacher, Department of Plant Pathology, Univ. California, Riverside, provided useful editorial suggestions. The author wishes to record here his sincere acknowledgements to those mentioned above.

RESULTS

1. Greening

(1) Symptoms and distribution

Based on field surveys, large numbers of sweet orange and mandarin budlines introduced from Pakistan or India in about 1967 showed severe zinc deficiency-like yellowing, vein chloroiss, leaf-blotch mottling, sparse leaf, dieback and poor fruit setting (Fig. 1, 2). The leaf symptoms, however, differed from typical zinc-deficiency in that small green-blotches occurred between veinlets (Fig. 2). According to observations by citrus agronomists, the leaf chlorosis was mild on the new shoots during a low temperature season, but the symptoms became severe with increase of temperature. Furthermore, affected leaves dropped prematurely in high temperature seasons. Those trees were also stunted and declining. The symptoms were similar to those of "greening" disease in South Africa described by Oberholzer *et al.* (19) and "die-back" in India (12).

Futher surveys revealed the presence of leaf-blotch mottling on native lemon (Fig. 3) and Kagzi lime (*C. aurantifolia* Swing.) trees. These trees were usually propagated by layering or cutting and were vigorously grown while young. However, leaf chlorosis and severe defoliation occurred during high temperature seasons and the trees deteriorated with the advance of gumming or dieback. A few 1 to 2 year-old seedling trees of pummelo (*C. grandis* Osb.), Natsudaidai (*C. natsudaidai* Hay.), rough lemon (*C. jambhiri* Lush.), and sour orange (*C. aurantium* L.), which were potted and placed behind the CVSRC building, also exhibited the symptoms.

Among the introduced cultivars from Japan since 1978, a few trees of navel orange (*C. sinensis* Osb. var. *brasiliensis* Tan.), Seminole tangelo, Iyo (*C. iyo* Hort. *ex* Tan.), Natsudaidai, Hirado buntan (*C. grandis*), Yuzu (*C. junos* Sieb. *ex* Tan.) and satsuma mandarin (*C. unshiu* Marc.), which were planted in some restricted fields near the trees with greening-symptoms or near the trees of native lemon and lime cultivars in CVSRC and ARS-jaintiapur, were also affected (Fig. 4, 5). Some of the trees developed the symptoms partially on one or two twigs. The affected trees observed are shown in Table 1.

(2) Collection of the vector

South African greening disease agent is transmitted by citrus psyllid, *Trioza erytreae* Del Guercio (16). In Asia another citrus psyllid, *Diaphorina citri* Kuwayama, is the effective vector for greening disease (8). Presence of *D. citri* in Bangladesh was previously recognized (Shahana Begum, personal communication). The author also observed both adults and nymphs of the psylla sucking on the potted Seminole tangelo, Natsudaidai and pummelo trees placed behind the CVSRC building in early July (Fig. 6). Thereafter, the psyllid was found on various citrus plants not only in the CVSRC main center but also in the Ishurdi area. The psyllid was also observed on trees of Kamini or Orange Jasmin (*Murraya paniculata* (L.) Jack), which is a popular ornamental tree in the country (Fig. 7). The psyllid multiplied abundantly on newly developed and immature shoots of those plants. The adults were occasionally found feeding on African marigold (*Tagetes erecta* L.) and Zinnia (*Zinnia* sp. L.) plants, which were planted under the Kamini tree. Those herbaceous plants also exhibited leaf mottling and irregular leaf shape.

The specimens in spirits were identified as *Diaphorina citri* Kuwayama by Dr. Y. Miyatake, Osaka City Museum for Natural History. And the ratio of the male to female was determined as one to one by Mr. K. Takagi, Kuchinotsu Branch, Fruit Tree Research Station. The adults and nymphs of *D. citri* collected in the OVSRC field are shown in Figs. 8 and 9.

(3) Electron microscopy

Midveins or petioles of immature and fresh leaves were collected from several citrus plants showing typical symptoms of greening disease and from some symptomless plants. The samples were cut into 1 mm lengths using a razor blade and immediately pre-fixed with 5 % glutaraldehyde (GA) solution. Some samples were immediately post-fixed with 1 % osmium tetraoxide (Os) solution and then embedded into Spur resin according to standard procedures. Other samples were brought to Japan in GA solution, and then post-fixed with Os solution followed by embedding into Spur resin. An ultrathin section was double stained with ulanium acetate solution for 40 minutes and with Reynold's lead nitrate solution for 15 minutes. H-300 electron microscope, Hitachi Co. Ltd., was used for an observation.

The presence of "Gracilicute-like bacterium" (GLB) in sieve tubes (Fig. 10-a) or surrounding cells of the plants are listed in Table 2. GLB was characterized by variable sizes ranging 100-1,000 x 200-2,000nm (Fig. 10-f), irregular shape such as ovoid, sphere or tube (Fig. 10-d), electron-dense fibril-nucleus indicating procaryotic organism (Fig. 10-c), and with having an envelope of about 20 nm in thick (Fig. 10-b). GLB was observed not only in vacuoles but also in the cytoplasm. The GLB structure was not detected from the control samples collected from a healthy citrus plant held in a glasshouse in Japan. These characteristics of GLB are identical with those associated with greening disease agent in South Africa and India (3).

Typical GLB structures were also observed in the sieve tubes of Elachi lemon, seedless lemon, Kagzi lime and Sarbati sweet lime (Fig. 10-c, d, f), which were symptomless or showing mild symptoms in Bangladesh. In addition, symptomless Kamini trees were shown to contain GLB (Fig. 10-e). Yoshida navel and seedlings of pummelo and Yuzu which had been free of the disease before planting in the field contained GLB.

2. *Gumming and dieback*

Large numbers of native lemon and lime cultivar tree were affected by a gumming problem and dieback in Bangladesh. Some parts of the main trunk and branches turned dark brown and the surface became longitudinally cracked. During the rainy season, the lesions were gumming (Fig. 11). Diseased trees on which lesions were extremely extended deteriorated and finally died. If twigs were severely affected, twig dieback occurred. When the surface of the lesion was scraped with a knife, infected and decayed tissue was observed to be distinctly surrounded by healthy tissues (Fig. 12). Symptoms of scaly bark or extended browinsh staining of the wood, which characterized foot rot disease caused by *Phytopthora*, was not observed.

Initial lesions were found on the surface of a smaller branch or twig as a pale brown blotch and pycnidia were formed there (Fig. 13). Based on microscopic examination of transverse sections of the lesion, complete pycnidia in a subepidermal stroma (Fig. 14), and broadly ellipsoid, rounded end, hyaline and 1-cell pycnospores without appendage and with short condiophore were observed. Thus, the fungus *Dothiorella* sp. may have been present (2). In the diseased tissues, there were 2 to 3 layers of protection tissues each consisting of lignified cells and divided cells. Internal tissue below the deepest protection-layer was still healthy; however, outer parts of the layer collapsed with penetration of abundant hyphae (Fig. 14).

Sweet orange and satsuma mandarin trees planted in CVSRC showed another type of gumming, which initiated from an injured tissues, i.e. pruning (Fig. 15).

Isolation and identification of the causal fungus from those lesions, and confirmation of its pathogenicity are currently being undertaken by the plant pathologists in Bangladesh.

3. Tristeza and stem pitting disease

(1) Symptoms and distribution

Field surveys showed that large numbers of citrus trees except for some budlines introduced from Japan were free of stem-pitting (SP). Budlines exhibiting SP symptoms were as follows: Fukuhara orange (sweet orange, SW), Kin-kunenbo (SW), Kanton orange (SW), O-mishima navel (SW), Thornton tangelo, Batangas, All Spice, Tachibana mandarin and red blush grapefruit. Some trees of Kagzi lime, one of the native varieties, showed mild symptoms consisting of a slight vein-clearing.

Several trees of sweet orange and satsuma mandarin grafted on pummelo or lemon rootstock were severely stunted and yellow. The symptoms differed from those of greening disease. Similar trees grafted on tristeza-resistant rootstock such as Yuzu and mandarin were vigorously grown. These suggest the presence of seedling yellows tristeza virus in the scion. However, some trees grafted on pummelo rootstock were still vigorous in ARS-Jaintiapur. These were propagated from trees which had been previously planted in the field 1-2 years before.

Citrus brown aphid (*Toxoptera citricida*), a primary vector for CTV, was found in the fields of CVSRC-Dhaka and ARS-Jaintiapur. However, it did not seem to be very abundant in the country.

(2) Detection of tristeza virus by ELISA

Citrus tristeza virus (CTV) and its vector are widespread in Asia including India (20). Therefore, a relationship between the citrus plant disorder and the presence of CTV was investigated.

For detection of CTV, enzyme-linked immunosorbent assay (ELISA) was first applied for citrus in Bangladesh. The anti-CTV serum used for ELISA was prepared in Japan. The antigen used was purified CTV-SP particles collected from hassaku dwarf affected fruit. Immature leaves were collected from 2 trees of each budline or cultivar planted in CVSRC-Dhaka and ARS-Jaintiapur. Each 0.5 g of the sample was macerated in a mortar with 5 ml PBS solution added with 0.05 % Tween-20, 2 % polyvinyl pyrrolidone and 0.05 % thioglycolic acid, and then centrifuged at 3,000 rpm for 15 minutes. The extract was used for the ELISA. Similar extract of tissue collected from a virus-free seedling was used for the control. The color reaction was determined visually after 2-hours incubation at room temperature.

Results showed that CTV was widespread in the trees of local lemon, acid lime and mandarin (Table 3). In contrast, large numbers of the trees propagated from introduced budlines from Pakistan or India were still free of CTV. Those trees were severely affected with greening disease. The large numbers of trees introduced from Japan since 1978 were carrying the virus except for a few budlines such as Natsudaidai and pummelo. This finding indicates there is no relation between greening-like disorder in Bangladesh and the presence of CTV.

4. Other diseases

Bacterial canker disease was prevalent on lemon, lime, sweet orange, tangelo and Natsudaidai trees. Fruit, twigs and leaves were severely infected, and this occasionally caused defoliation, fruit drop and dieback. Heavy and frequent rainfalls were a major factor for epidemic of the disease because of a rain-born pathogen. Strong wind, especially accompanied by rain, was also important, because it not only produces abundant wounds on the host plant but also disperses the causal bacteria from inoculum sources. Wounds caused by citrus leaf miner were significant. These wounds were easily infected by the pathogen.

Greasy spots, scab and melanose, which are fungul diseases, were also observed; but they did not seem to be very important in the country.

DISCUSSION

Greening disease, first called yellow branch, has been known since 1929 in South Africa. The disease was originally believed to be of virus-origin. Some evidence of transmission of the causal agent by grafting was reported in 1955 (7). The citrus psylla (*Trioza erytreae* Del Guercio) was reported to be the vector of greening disease agent in South Africa in 1964-1965 (16, 22). Lafleche and Bove (14) demonstrated the presence of mycoplasma-like organisms (MLO) in the sieve tubes of greening-affected plants. Soon after, the envelope of the organisms was found to be 15-25 nm wide, too large to be a simple-unit membrane. This indicated that the organism associated with greening disease was different from ordinary MLO (3, 21). Bove *et al.* (5) proposed a more appropriate term "Gracilicute-like bacterium" (GLB) for the organism associated with greening disease. Moll *et al.* (17) described the transmission mechanics, that is, the organism is ingested by the phloem feeding adult psylla and penetrates the gut during a latent period of about 21 days after which it multiplies in the hemolymph, penetrates the salivary glands and is injected with saliva into the next plant during feeding. This suggests that once infected, the vector can transmit the GLB until it dies.

Fraser and Singh (12) reported in 1966 that citrus dieback disease in India might be caused by the pathogen responsible for greening disease in South Africa, based on the similarity of symptoms, host range and reaction. Afterwards, Capoor *et al.* (8) showed the transmission of greening disease agent in India by the phsyllid vector, *Diaphorina citri* Kuway. Similar diseases recognized in the Asian countries such as citrus leaf mottling in the Philippines (15), citrus yellow shoot or yuan lung bing in the southern parts of China (25), likubin in Taiwan (23) and citrus vein phloem degeneration (CVPD) in Indonesia (24) were also shown to be transmitted by *D. citri* Microorganisms similar to those associated with South African greening disease were detected in the affected trees with Indian dieback and Philippine leaf mottling (21), Chinese yellow shoot (25), likubin (10), and in the salivary glands of *D. citri* (11). These diseases are considered to be a form of greening disease. However, characteristics of Asian greening disease differ from those of African type not only in the vector but also in the sensitivity of symptom expression to temperature. Trees affected by Asian greening disease develop severe symptoms at high temperatures (27-32°C) as well as at low temperatures (22-24°C), whereas the African greening-infected plants remain symptomless at the higher temperatures (4).

Up to the present, Asian greening disease and the vector *D. citri* have been also reported from Pakistan, Nepal, Thailand, Malaysia (9), Reunion (1), and Saudi Arabia (6). The disease is prevalent in India, particularly in the Punjab, Northern and Central India, Assam and Orisea, South India, Bihar, West Bengal, Sikkim and Uttar Paradesh (20). The present report describes the prevalence of the disease and the vector in Bangladesh.

Greening disease in Bangladesh is common not only on the introduced cultivars from Pakistan or India, but also on the native varieties of lemon and lime. Furthermore, sweet orange or mandarin trees disappear in the country as a productive crop, while lemon, sour lime or pummelo trees are commercially cropping. This suggests that greening disease-susceptible plants have been destroyed, whereas greening-tolerant plants remain. Pummelo is susceptible when young; however, it becomes tolerant with maturity. Kamini (*M. paniculata*) is also infected with the pathogen, but it seems tolerant. Thus, these tolerant plants are carriers of the causal GLB and provide a source of inoculum. In addition, the psyllid vector multiplies abundantly on those plants. *M. paniculata* seedlings can be infected with the pathogen by *D. citri* (24); however, this is the first report of the presence of GLB in this host.

The disease is now spreading into the trees of cultivars introduced from Japan since 1978, particularly into sweet orange, navel orange, tangor, tangelo and satsuma mandarin trees. These trees will follow a similar decline as the formerly introduced cultivars unless effective disease-control-measures are immediately implemented.

Gumming or dieback is most destructive on lemon or lime trees in Bangladesh. The disease seems to be caused primarily by a fungus, probably by *Dothiorella* sp. However, the anatomical study revealed the presence of several protection layers in tissues within the lesion. The upper layers of the protection tissue were broken with penetration of hyphae. The findings suggest a mechanism of lesion development as follows: Bark tissue is infected by the causal fungus, immediately followed by the formation of a protection layer below the infection sites. However, the layer is penetrated by the hyphae and broken after a period when the tree weakens. Thus, the inner tissues become infected and another protection layer is formed below the newly infected tissues. Continuous repetition of those make lesion extend deeply and widely. Therefore, deterioration of tree growth is the most promotive factor for development of gumming disease. Observations by citrus agronomists imply that lemon or lime trees grow vigorously during the lower temperature season; however, their shoots become yellow and mottled, i.e. greening disease, and finally defoliate during the higher temperature season. Therefore, the presence of greening disease must be the main factor in weakening the tree and permitting development of gumming disease in Bangladesh.

Citrus tristeza virus (CTV) and its vector are widespread in Bangladesh. However, severe symptoms such as stem pitting, dwarfing and decline were not observed on native varieties and Pakistan-or India-introduced cultivars. These include not only highly susceptible plants such as sour lime but also highly susceptible scion-rootstock combinations such as sweet orange on pummelo. In addition, large numbers of the trees are free of CTV, even though they are yellowing and declining. Thus, severe strains of CTV, such as seedling yellows CTV, is inactivated in the country. This might be due to the high temperatures. Tristeza virus seems not so important as greening disease in Bangladesh. However, Fraser et al. (12) suggest that dieback is due to combination factors in India, especially related to the presence of severe tristeza isolate. Therefore, it would be better to use a tristeza-resistant rootstock such as rough lemon for sweet orange or mandarin scion.

CONCLUSION

The main causal factor of the citrus plant disorder in Bangladesh, including gumming or dieback, is greening disease caused by "gracilicute-like bacterium". The agent is transmitted by the citrus psyllid (*Diaphorina citri* Kuway.). Development of citrus production in the country will not be possible unless the disease is satisfactorily controlled. Considering the recommended programs of integrated control of the disease in India (18), in China (25) and in the Philippines (13), a program to control the disease in Bangladesh is proposed as follows:

1. Grow nursery plants in a screenhouse to prohibit sucking of the psyllid vector, or in a disease, or vector-free area where greening-carrier plants had been eradicated.

2. Use greening disease-free budwood obtained by immersing budsticks into 1,000 μg/ml tetracycline hydrochloride solution for 3 hours or by incubation of budsticks in a moisture-saturated air at 49°C for 50 minutes, at 47°C for 4 hours or 45°C for 6 hours.

3. Use a tolerant rootstock like sweet lime.

4. Control citrus psylla by insecticide, particularly when new flushes are present. Sprays with dimethoate, methasystox, dimecron or nuvacron are effective.

5. Remove greening affected trees immediately after symptom appearance. All steps from 1 through 5 are necessary for controlling the disease.

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Table 1. List of citrus cultivars or budlines exhibiting greening symptoms in Bangladesh

Origin of plant	Symptom intensity			
	Non	Mild	Severe	
Native var.	Kagzi L (D) Elachi LM	Seedless LM Kagzi L (I) Adajamir H	Kasia M (J)	
Introd. var. from Pakistan or India	Sarbati SL	Valencia SW Jaffa SW Washington N Dancy TG	Featureless M Satsuma M Nagpuri M Ruby Red SW Red Blood SW	Kinnow M Mosambi SW Suckery Pineapple SW Decibre SW
Introd. var. from Japan	Large No. of trees includ. all introd. cultivars	Satsuma M (D, J) Natsudaidai (D, J) Hassaku (P) Yuzu (D) Eureka LM	Yoshida N (D, J) Oomishima N (D, J) Iyo (J) Seminole (P) Fukuhara SW (P)	 Hirado (D)
Seedlings	Kasia M (J)	Pummelo (P) Natsudaidai (P) Sour orange (P) Rough lemon (P)	Pummelo (D)	

L, acid lime; LM, lemon; SW, sweet orange; M, mandarin; N, navel orange; H, *C. histrix;* SL, sweet lime; TG, tangerin.

A letter in the parenthesis means as follows: D, in a field of CVSRC, Dhaka; *I, Ishurdi area; J, ARS- Jaintiapur; P, potted trees in CVSRC, Dhaka.*

Table 2. List of plants examined by electron microscopy for presence of gracilicute-like bacterium (GLB).

Examined plant	Collection place	Symptom intensity	Presence of GLB
Decibre SW	No. 2 field, CVSRC	severe	positive
Yoshida NV	A field near to CVSRC	severe	positive
Elachi lemon	ditto	non	positive
Seedless lemon	ditto	mild	positive
Kagzi lime	ditto	non	positive
Sarbati SL	ditto	non	positive
Pummelo SD	ditto	severe	positive
Yuzu SD	ditto	severe	positive
M. paniculata	Banani, Dhaka	non	positive
Grapefruit SD	Kuchinotsu, Japan	non	negative

SW, sweet orange; NV, navel orange; SL, sweet lime; SD, 3-year-old seedling.

Table 3. Detection of citrus tristeza virus from various citrus plants in Bangladesh by ELISA.

Origin	CTV-negative plant	CTV-positive plant
Native var.	Kasia M (J) some Elachi LM (D) *M. paniculata* (DB)	some Kasia M (J), Elachi LM (D) *C. histrix* (J), Kagzi L (I, D, J) Seedless LM (J, D)
Introduced var. from Pakistan	Valencia SW (D) some Pineapple SW (J) unknown SW (D) Mosanbi SW (D, J) Decibre SW (D) Ruby Red SW (D) Nagpuri M (D), Suckery (D) Kinnow M (J), Sarbati SL (D)	Jaffa SW (D, J) Pineapple SW (J) Blood Red SW (J) Multa SW (J) Satsuma M (D) Featureless Early M (D)
Introduced var. from Japan	some Yoshida NV (J) some Aoshima SM (J) Tosa Buntan P (D) Shutou SO (D), Banokan P (D) Anseikan P (D) Shin-amanatsu N (D) Kinkoji (D) some Banpeiyu P (D) some Lisbon LM (D)	Other trees including sweet orange, navel orange, lemon, Hassaku, Natsudaidai, Pummello or buntan, grapefruit Tachibana, satsuma mandarin, tangerin, Hyuganatsu, Iyo, tangelo, tangor,
1-2-year-old seedling	Pummelo (D), SO (D) N (D), Rough Lemon (D)	

L, acid lime; LM, lemon; M, mandarin; N, Natsudaidai; NV, navel orange; P, Pummelo; SL, sweet lime; SM, satsuma mandarin; SO, sour orange; SW, sweet orange.

A letter in the parenthesis means the place where a sample was collected: D, CVSRC-Joydebpur; DB, Dhaka, Banani; I, CVSRC substation-Ishurdi; J, ARS-Jaintiapur.

Fig. 1

Severe yellowing and dieback of ruby red sweet orange/ pummelo tree, at CVSRC maincenter, Joydebpur.

Fig. 2

Chlorosis and irregular blotch mottling of ruby red sweet orange leaves, at CVSRC maincenter.

Fig. 3

Vein chlorosis and blotch mottling of seedless lemon leaves, at ARS, Jaintiapur.

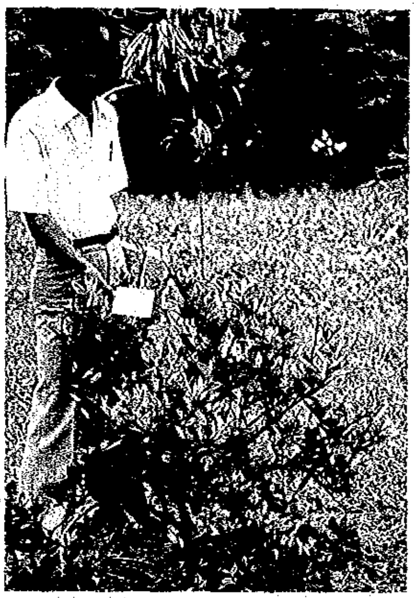

Fig. 4

Greening-symptoms observed on a Yoshida navel/trifoliate orange (TRIF) tree which was introduced from Japan, originally free of greening disease when planted in May, 1981, at ARS, Jaintiapur. This indicates natural infection of the disease in the field.

Fig. 5

Typical greening symptoms observed on a Hirado buntan/TRIF tree introduced from Japan, at CVSRC maincenter. This also indicates natural infection in the field.

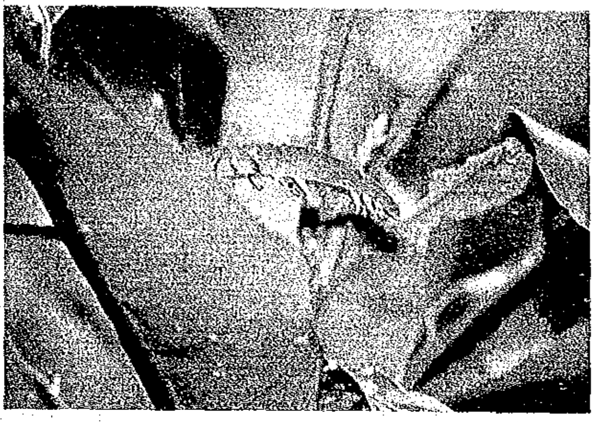

Fig. 6

Sucking of the citrus psylla vector, *Diaphorina citri* Kuway., on a Natsudaidai potted tree, at CVSRC maincenter.

Fig. 8

The adult of citrus psyllid collected in CVSRC maincenter.

Fig. 7

The psyllid vector on Kamini tree, *Murraya paniculata*, at Banani, Dhaka.

Fig. 9

The nymphs of citrus psyllid collected in CVSRC maincenter.

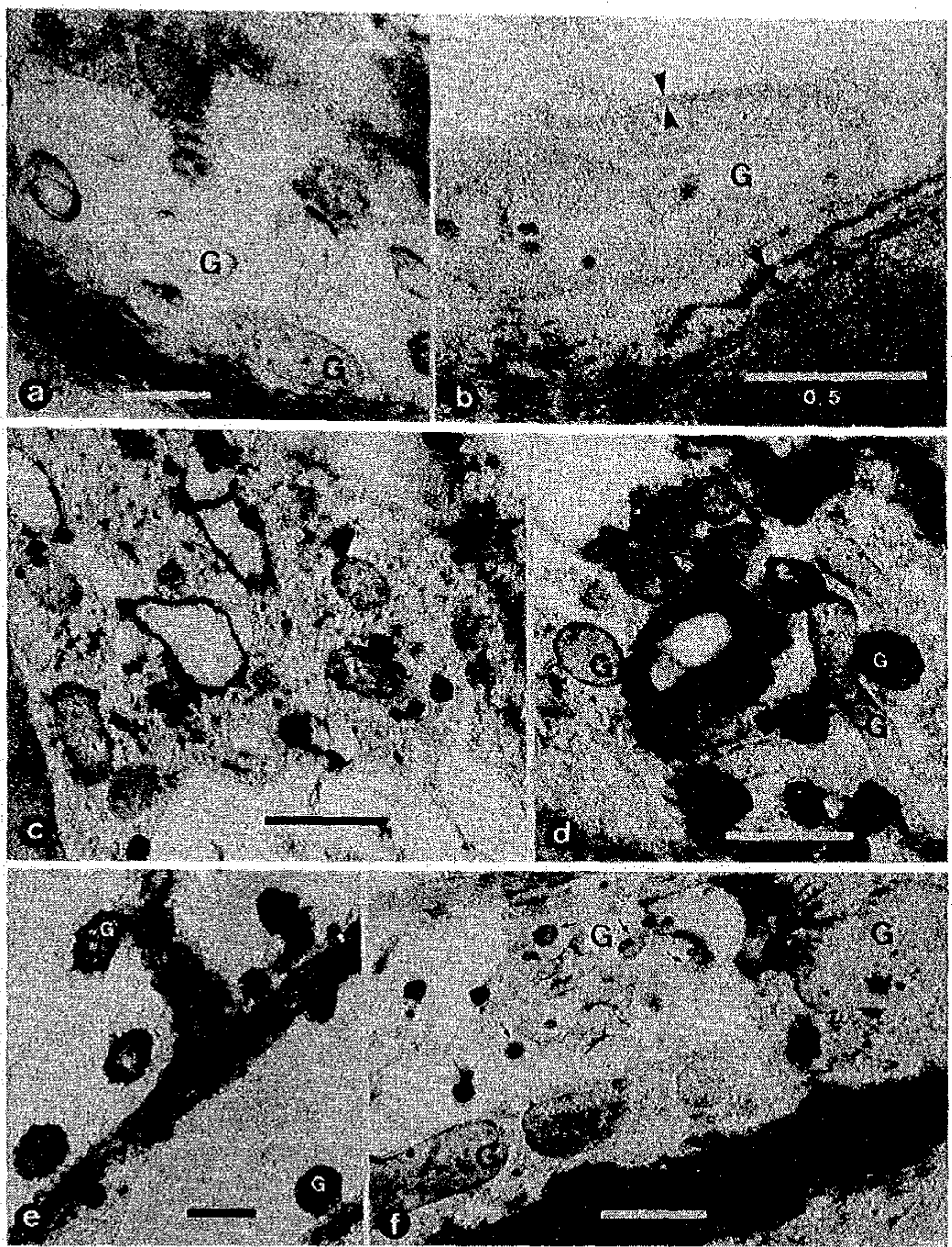

Fig.10

Electron micrographs showing the presence of "gracilicute-like bacterium" (GLB), probably greening causal agent, in various citrus or citrus relative plants collected in Bangladesh.

a. GLB in a sieve tube of Decibre sweet orange showing typical greening symptoms.
b. Enlargement of a lower part of the plate a, indicating 23 nm in thick of GLB-envelope and 10 nm in thick of the host plant plasmalemma.
c. GLB within cytoplasm of a sieve tube of Elachi lemon, a symptomless carrier.
d. Various shapes of GLB within a sieve tube of seedless lemon.
e. GLB within a sieve tube of Kamini plant, *M. paniculata*, a symptomless carrier.
f. Various shapes of GLB in a sieve tube of Sarbati sweet lime, a symptomless carrier.

G = "Gracilicute-like bacterium".

Bar represents 1,000 nm except for the plate b.

Fig.11
Dothiorella associated gumming on a seedless lemon tree, at CVSRC maincenter.

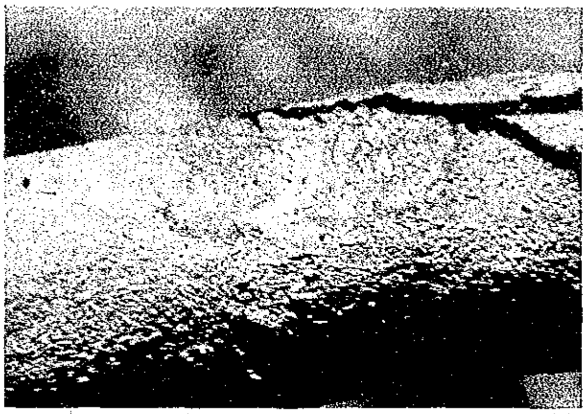

Fig. 13
An initial lesion of *Dothiorella* gumming on a Kagzi lime tree.

Fig. 14
A transvarse section of the initial gumming lesion developed on a seedless lemon tree, showing the presence of 3 protection-layers.

Fig. 12
Diseased tissue scraped with a knife, showing dark brown and deeply infected tissue distinctly surrounded with healthy tissues.

Fig. 15
Another type of gumming or dieback developed on a sweet orange tree, showing gum oozing from the wounded part due to pruning.

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