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"RESEARCH ON CITRUS GREENING VECTOR IN POKHARA VALLEY"

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1. INTRODUCTION

Agroclimatic conditions of Nepal are quite suitable for citriculture. Citrus are native to Nepal and they are grown traditionally for a long time. They are cultivated in 45 districts out of 75 in the whole mid hill range of the country. They cover 7850 ha of cultivated land with annual production around 4500 mt. Government has a target to increase the area under citrus up to 13300 ha by the end of this Seventh Five Year Plan 1985-1990. However national citrus fruit production is insufficient to fulfil even the internal demand due to comparatively low productivity (8-9 mt/ha).

His Majesty's Government of Nepal has set up a "National Citrus Development Program (NCDP). A Juner (orange) Development Project has been started in Sindhuli and Ramechhap districts since last 2 years. Recently a "Hill Fruit Development Program " is being implemented in the 11 hill districts of Eastern Development Region under the financial support of Asian Development Bank. This project is expected to cover 4800 ha citrus orchards. Likewise another project is under feasibility study for the citrus development in 19 districts of other Development Regions. Besides that some priority areas have been selected for citriculture. Farmers of these areas have got some facilities of getting loans on low interest and planting materials free of charge. Considering the economic importance of citrus, government is doing all these efforts with huge investment.

Citrus Greening Disease (CGD) is one of the serious problems for citrus fruit production in Nepal. The disease is systemic, so surface protection is not possible. It is transmitted by insect vector and by grafting hence there is a chance of spreading the disease both in the field and nursery. It has long incubation period and no quick and reliable as well as practical technique has been developed yet for early diagnosis. All these facts dictate the complication in developing and implementing any controlling measures against the CGD.

Royal Nepal Academy of Science and Technolgy (RONAST) places special emphasis on wide consultation with scientists and technologists in formulating its plans and programs. Accordingly RONAST had constituted 23 Working Groups in different field of science & technology by involving a large number of scientists and technologists of the country to get valuable suggestions for the development of s&t capability of the country. Reports submitted by these working groups were discussed in a seminar participated by more than 350 scientists and technologists who urged RONAST to implement the recommendations given by the working groups. RONAST is already implementing some of those recommendations within its limited physical and man power facilities. Working group on Agriculture, considering the burning problem in citriculture, had pointed out the immediate need of research on CGD. This research project is one of the follow up activities of such recommendations initiated by RONAST. It prepared a research proposal entitled "Study on Citrus Greening Vector in Pokhara Valley" and submitted to USAID for PSTC (Program in Science & Technology Cooperation) grants. This project was approved and recieved US$ 15000 under innovative scientific research program of PSTC in 1985. As one of the policy of RONAST is to conduct collaborative research, it involved Department of Agriculture HMG/Nepal. A memorandom of understanding [effecting from 15 July 1985 (1 Sravan, 2042)] was made with the Department of Agriculture to conduct jointly this research project. Accordingly one plant pathologist, field and laboratory facilities of Horticulture Research Station, Pokhara were alloted for this research from Department of Agriculture.

2. LITERATURE REVIEW

Greening disease was reported as a new disease of citrus from South Africa by the end of 1950s. However this disease was already known in 1920s as yellow branch disease in South Africa and yellow shoot disease in China (Broadbent 1983, Oberholzer etal 1965). At present this disease has been reported from several countries of Asia, Africa and islands of Indian Ocean(table1) causing severe damage to citrus production.

Table 1

Distribution of Greening Disease and its Vector in different countries of the world

SN	Country	Disease with Synonyms	Vector Species	Authors reporting the disease/Vector
1.	Bangaladesh	Greening	*D.citri*	Catling 1978
2.	China	Yellow Shoot	*D. citri*	Lin 1956, Su et al 1972
3.	India	Citrus decline	*D. citri*	Fraser et al 1960, Capoor et al 1967
4.	Indonesia	Citrus vein phloem degeneration	*D. citri*	Tirtawidjaja 1964, 1983
5.	Malaysia	Greening	*D. citri*	Catling 1968, Euno 1978
6.	Nepal	Greening	*D. citri*	Knorr et al 1970, Rana et al 1964
7.	Pakistan	Greening	*D. citri*	Knorr et al 1970
8.	Phillipines	Leaf mottling	*D. citri*	Salibe et al 1966, Martinez et al 1967, Celino et al 1969
9.	Saudi Arabia	Greening	*D. citri* *T. erytreae*	Bove 1986
10.	Yemen Arabic Republic	Greening	*D. citri* *T. erytreae*	Bove 1986
11.	Taiwan	Likubin	*D. citri*	Catling 1970, Su et al 1972
12.	Thailand	Greening	*D. citri*	Thrower 1968, Schwarz et al 1973
13.	Angola	-	*T. erytreae*	Aubert 1983
14.	Cameroon	-	*T. erytreae*	Aubert 1983
15.	Ethiopia	Greening	*T. erytreae*	Aubert 1985
16.	Kenya	Greening	*T. erytreae*	Aubert 1985

17.	Ruanda	-	*T. erytreae*	Aubert 1983
18.	S. Africa	Greening	*T. erytreae*	Oberholzer et al 1965
19.	Sudan	-	*T. erytreae*	Aubert 1985
20.	Swaziland	-	*T. erytreae*	Aubert 1985
21.	Tanzania	Greening	*T. erytreae*	Aubert 1983
22.	Uganda	-	*T. erytreae*	Aubert 1985
23.	Zaire	-	*T. erytreae*	Aubert 1985
24.	Zimbabwe	Greening	*T. erytreae*	Aubert 1985
25.	Madagaskar	Greening	*D. citri* *T. erytreae*	Bove et al 1968 a, Aubert 1984 a
26.	Mauritius	Greening	*D. citri* *T. erytreae*	Bove et al 1968 a, Aubert 1984 a
27.	Reunion	Greening	*D. citri* *T. erytreae*	Lafleche et Bove 1970 b, Aubert 1983
28.	Seychelles	Greening	*D. citri* *T. erytreae*	Aubert 1984 b

Some examples of loss due to CGD are cited here. More than 16000 ha and 900 ha citrus orchards were destroyed since 1958 in Philippines and China respectively. It is estimated that more than 15000 trees are infected in South Africa and 40- 70 % trees are infected in Nepal and Thailand (Altomirano 1976, Broadbent, 1983, Knorr et al 1970 Regmi 1982).

2.1 Symptoms

The name greening was given because the fruits maintained green colour even after maturity. However the symptoms of greening vary depending on the agroclimatic conditions, virulence of pathogen, and genotype of citrus. Visual symptoms of CGD appear with leaf mottling developing irregular patches between the main vein resembling zinc defeciency like symptoms leading to the general chloroz, leaf drop and even apical twig die back. Infected trees have stunted and open growth, sparse foliage, yellow branch aspect and severe decline in some cases. Fruits are under developed, lopsided with aborted seeds (Bove et al 1981).

2.2 Pathogen

Schwarz (1964) first confirmed infectious nature of CGD on the basis of graft transmission of the disease. The greening organism (GO) was supposed to be virus. This theory became popular upto 1970. Lafleche and Bove (1970) reported the mycoplasmalike nature of GO while studying the structure under electronmicroscope. Positive effect of tetracycline treatment on greening infected trees also lead to relate it with mycoplasma like organism (MLO) (Martinez et al 1970, Su et al 1976). But immediatly Moll and Martin (1974) presented evidences that the GO had a double

trackmembrane like cell wall in addition to its inner cytoplasmic membrane and suggested the term Bacteriumlike Organism (BLO). Bove and Saglio (1974) confirmed three layered structure of outer membrane of GO by screening under electron microscopy. Garnier et al, observed peptidoglycan (PG) layer in the envelop of GO by treating with papain. This layer was very similar to that of *E. coli* - a gram negative bacterium and was removed by lysozyme treatment (Garnier etal 1984). Therefore they have suggested GO to be a gram negative bacterium, although Bove et al (1980) had suggested the term "Gracilicute like organism" for it. But uptill now no scientific name has been given to it and scientists refer it simply as GO.

GO are pleomorphic cells (filamentious and rod forms) approximately of 3000x350nm size. They consist of two membranes, outer (envelop or cell wall) and inner (cytoplasmic) cell wall. Cell wall 20-30 nm in width is comprised of three layers ; a dark electrone obserbing inner layer (8 nm), a dark outer layer (8nm) and intermediate clear, electrone transparent layer (5-15 nm) containing peptidoglycan. Gernett (1984) also has reported that she could culture GO in MIG media, the colonies of which contained filamentiuos and rod shaped organisms of similar size as mentioned above.

2.3 Vector

McClean and Oberholzer reported *Trioza erytreae* (Del- Guercio) as citrus greening vector for the first time in 1965 from South Africa. Two years later Capoor et al (1967) reported Asian greening vector - *Diaphorina citri* (Kuw). Only these two vectors of CGD have been reported so far. Geographical distribution of these two vectors is given in table 1. It can be seen that *T. erytreae* is dwelling with or without greening in African continent where as *D. citri*is in Asia. Both vectors have been reported from islands of Indian Ocean and Kingdom of Saudi Arabia (Bove 1986)

Intensive works have been done on different aspect of vector and its relationship with disease by several authors. Aubert (1986) has summerised all these studies and has prepared a comprehensive report on these psyllids (Aubert 1986). Both citrus psylla - *D. citri* and *T. erytreae* belong to the super family of Psylloidae of Psyllidae family (Homoptera). They have three stage life cycle (Egg-Nymphs-Adults) with 5 instars of nymphs which completes its life cycle within 15-20 days in hot and dry and 40-45 days in cold seasons. They don't undergo diapause. They have ventrally elongated mouth parts which is adopted for sucking the plant vessels. *T. erytreae* does not tolerate hot and dry climates while *D. citri* prefers it. Accordingly the first one is the vector of South African strain and second one is that of Indo-Phillipine strain of CGD, although experimentally it has been proved that each of them are capable of transfering both of these strains (Massonie et al 1976).

Although there is little information on the vector pathogen relationship, it is admitted that adult psylias take up the greening organism (GO) from infected plants; the GO multiplies subsequently with in the inesect body. Due to the concentration of GO seen in the salivery glands it is thought CGD is transmitted through the secretion of salivery ducts (Aubert, 1986).

These psylias are host specific. Aubert (1986) mentioned 17 host plants of *T.erytreae* and 13 host plants of *D.citri*. He also categorised the host plants into preferential, common and alternate. Psyllids only feed on alternate host plants without laying eggs and developing nymphal stage. All the host plants are confined to the species of Rutaceae family only. Preference of host plants is determined by their frequency of

flushing rythms. Population of psyllids is directly correlated with the flush on which psyllids feed and breed (Capoor 1967, Moinshah 1984, Aubert 1986).

2.4 Natural Enemies

Calling (1970) studied the bionomics of these psyllids and made important notes on the influence of natural enemies to reduce their population. Several authors also have reported the presence of natural enemies belonging to Syrphids, Coccenelids, Lacewings as well as spiders and mites as the predators of psyllids but the most effective predator was found to be *Chilocharus nigritus* belonging to Coccinellidae family (Coleoptera).

Parasites of these psyllids have been found to be more effective than the predators due to their high specfic nature in parasitising only certain species of psyllids. Three eulophid exoparasites of *T. erytreae* viz *Tetrastichus dryii*, *T. sicarius* (Waterstone) and a new *Tetrastichus* spp. have been reported (Aubert 1986). They attack 3rd, 4th and 5th instar nymphs. Likewise one encyrtid endoparasite - *Psyllaephagus pulvinatus* attacking the 2nd,3rd,4th and 5th instar nymphs is also reported from South Africa. Regarding the parasite of *D. citri* only two species of parasitoid wasps have been reported. One of them is eulophid ectoprarasite *Tetrasticus radiatus* (Waterstone) and the another one is encyrtid endoparasite - *Diaphrencyrtus alegharensis* (Shaffee et al). Recently a primary parasite of *D.citri* was discovered in Fuzhou, China. Noyes has identified this parasite as *Tamariria near radiatus*. Further studies on this parasite are continued (Aubert 1988, personal communication).

However only T.dryii against *T.erytreae* and *T.radiatus* against *D. citri* are being practically used for biological control in Reunion, Mauritius and Taiwan (Etienne et al 1980, Joomaye et al,1986,Shui-cheng et al 1988). The effectiveness of these parasites depends upon the presence of their hyper prarasites i.e. secondary and tertiary parasites. All together 11 species belonging to Eulophidae, Encyrtidae, Aphelinidae, Ceraphronidae and Pleromalidae have been found as secondary and tertiary parasites of *T. erytreae* while only *Marietta javenensis* (Howard) has been identified as secondary parasite of *D. citri* (Aubert 1986). Possibility of other hyper parasites of *D. citri* has been mentioned but they have not been yet taxonomically identified. Complex parasitism of *D. citri* is presented below.

Diaphorina citri	Ectoparasite (Eulophidae) *Tetrastichus radiatus* Waterston Endoparasite (Encyrtidae) *Diaphorencirtus alegarhensis* Shaffee et al.	*Marietta javenensis* How and other unidentified species.

2.5 Diagnosis

Development of a quick, easier and reliable method of diagnosis of CGD has got a significant importance because similar symptoms may be caused by various biotic and abiotic factors. They may vary depending upon the season, environment, strain of pathogen etc. More over the visual symptoms of CGD appear only at the fruit bearing stage of plant. Some of the common methods of diagnosis being used for the detection of CGD are summarised here.

2.5.1 Plant Indicator Method

This is common method used for the diagnosis of virus and virus like diseases of plants. The technique is based on the use of plants called plant indicators giving specific reaction in developing symptoms with the specific pathogen. Best plant indicator for CGD is sweet orange specially Madame Vinous and Mousambi but other citrus such as mandarin, tangelo and kagzilime also can be used (Calavan et al 1978). There are different procedures for greening inoculation to plant indicators such as bark and bud grafting, through dodder, and vector transmission. CGD is not sap transmissible. Plant indicators develop chlorotic patches on leaves as well as vein clearing and leaf drop symptoms within 3-4 months. This technique needs ideal and optimum conditions to grow plant indicators and it is time consuming too. However this is only the technique which is most reliable and most practical for the developing countries. But unfortunately even this facility is not available in our country.

2.5.2 Histochemical Method

The principle of this method is based on the fact that external symptoms of the disease should be associated with the anatomo- biochemical changes of plants. Iodine test is one of these methods which indicates the accessive accumulation of starch in leaves of diseased plants, Tirtavidjaja et al (1964) and Schnieder (1968) have developed histo-chemical method to index greening disease. They observed suberification of main ribs and proliferation of phloem in the anatomic section of infected leaves. But this method is not very reliable because similar histochemical changes may be caused by different diseases and abiotic factors, however it could be used for preliminary identification of the disease.

2.5.3 Thin Layer Chromatography (TLC) Test

Schwarz (1968) developed Thin Layer Chromatographic Technique, based on the presence of flourescence greening marker substance - Gentisoyl glucose in the extract of albedo and bark of 2-3 years old twigs of diseased mandarin and orange plants. This method was modified and used by Sharma etal (1974). Reliability of this technique is not very high due to its reaction on polyphenolic substance of plants which again may be caused by different factors and are not directly related with the pathogen or its product. However this is only the technique uptill now which can be afforted by developing countries like ours. This test can be done in any biological or chemical laboratories. TLC facilities are available at Horticulture Research Station, Pokhara.

2.5.4 Monoclonal Antibody Technique

This technique is based on the production of hybridoma clones secreting specific monoclonal antibodies (mAb) against the GO. The team of Prof. Bove has succeeded to prepare such mAb by using differential ELISA and immunoflourescence in screening hybridomas. Two such hybridoma clones were obtained by them (Garnier et al 1987). The mAb were specific to GO and gave positive reaction with sweet orange infected with Indian, Phillipine, Reunion as well as South African strains of greening. No cross reaction were seen with any other phloem limited prokaryotes tested nor with healthy plant material. This indicates the possibility of using mAb for detecting GO in citrus but frequently several sections have to be examined to get the positive reaction. This is due to the less content of GO in sieve tubes of citrus. Therefore French and American scientists are trying to improve this technique by combining polyclonal antibodies(pAb) and this mAb (Aubert 1988, personnal communication). It is hoped

that this technique will get due application in near future. RONAST can initiate testing of this technique in the context of Nepalese strain of greening.

2.5.5 Serological Method

This method is commonly used for the diagnosis of bacterial as well as virus diseases of plants, animals and man. It is based on the coagulating or precipitating property of serum with the specific pathogen from which the pathogen was prepared. Pure culture of pathogen is necessary for this purpose. Ghos etal(1971) and Sodhi et al (1973) reported that they succeeded to culture GO but Bove and other scientists failed to reculture it. So the culture of GO was still questionable. Later Garnett (1984) has discribed that she could culture GO in a complex media called MIG media. She and her collegue F. Duncan published that they have prepared antiserum by using GO which was isolated in vitro in MIG media. This antiserum prepared from chicken and rabbit gave positive reaction while conducting ELISA test with homogenated plant samples infected with greening disease. But Prof. Bove and his collegues could not get positive reaction of this antiserum with GO grown in periwinkle sieve tubes. However Agriculture Research Service, Beltsville of United States Department of Agriculture, has initiated research work on this aspect. It has also urged RONAST to conduct cooperative research in this field(Civerolo personal communication 1988). It is hoped that culture of GO will be successful and reliable serological method will be developed in near future for the diagnosis of CGD.

2.5.6 Electron Microscopic Technique

This is only method which is very quick, easier and gives the picture of size, shape and structure of pathogen. Several authors (Lafleche et al 1970, Moll et al 1973, 1974, Catling et al 1978) have used this technique for the detection of GO. The shape and size were determined by using this method. Three layerd membrane was also first pointed out on the basis of electronmicroscopic study of GO which refused previous thought of mycoplasmalike organism and lead to the bacterial nature of GO. It is most reliable technique but due to need of very sophisticated and costly equipment, developing countries like Nepal can not implement it.

2.6 Control

All the citrus species / cultivars are succeptible to CGD but sour citrus such as lime, lemon etc don't express visual symptoms. No resistant rootstock to CGD has been reported so far. Neither any chemicals have been found effective for the control of CGD. Although Tetracycline and Penicillin were found useful while applied by trunk injection and folier spray but they can't be used for commercial purpose (Bove et al 1980, Martinez et al 1970, Miyakawa 1979). Therefore only preventive measures are practical for this purpose. Accordingly supply of healthy planting material and control of vector are being implemented in different countries. Shoot tip grafting in vitro technique has been found to be very useful to eliminate various virus and virus like diseases (Navarro 1981). It is also supposed that this technique will be equally useful to eliminate the CGD. Thermotherapy of budwoods (hot water treatment at 45-47oC) also has been reported to be effective to eliminate the greening infection (Su et al 1976). These techniques can be used to establish Foundation Stock but not for the production of all the planting saplings. It is therefore more practical to select elite plants, eliminate greening and other virus disease infection by applying any of these above mentioned techniques, establish a foundation stock, maintain it by providing

proper plant protection management and supply the bud woods to the nurseries from these foundation stock for the mass propagation of planting material. This practice is commonly used in other citrus growing countries like Spain, South Africa, Reunion etc. Development of quick, easier and reliable diagnostic techniques will further open the possibility of on the spot selection of mother plants and consequently in supply of healthy planting material.

CGD can be controlled indirectly by controlling the population of vector. In this context biological control of vector has been successfully implemented in some countries, such as Mauritius, Reunion and Taiwan by using their natural enemies (Aubert 1985, Etienne et al 1980, Shui-chen 1988, Joomaye et al 1986). Further more Aubert has suggested integrated control of greening combining together the biological control of vector with the chemical one (Aubert 1986).

Review of the available literature suggest that CGD is and is being a serious problem for citriculture in several countries of Asia and Africa. Several scientists are engaged in research on different aspect of CGD. It has been felt that efforts of a scientist or a country are not enough to overcome this disease. In this respect some joint efforts have been initiated. They are;

1. Intercountry UNDP -FAO Regional Project of Governments of China, Indonesia, Malayasia, Phillipines and Thailand (1986-1990) on citrus greening disease.
2. Cooperative research on citrus greening disease -initiated by USDA, ARS, Beltsville with different related laboratories of the world including RONAST.
3. Scientific cooperation in combining mAb and pAb among French and American scientists.

Therefore it is suggested that we should have joint efforts to conduct research on different aspects of greening with the scientists of the country as well as with the scientists of SAARC countries in close contact with the leading laboratories and scientists of the world.

3 PROJECT OBJECTIVES AND RESEARCH ACTIVITIES

The objectives of this research project was to study the host plants and natural enemies of greening vector - *Diaphorina citri (Kuw)* with a view to workout the ecological and biological approach of citrus greening control. Following research activities were envisaged to achieve the above mentioed objectives.

1. Identification of host plants of *D.citri*.
2. Identification of citrus varietal succeptability to *D.citri*.
3. Life cycle study of *D.citri*.
4. Study the possibility of transoverial transmission of greening by *D.citri*.
5. Identification of predators of *D.citri*.
6. Trial on integrated citrus protection.

There was a provision of a study visit to Reunion at the first stage of the project. During our study visit to Reunion (January, 1986) we had discussed with Prof.J.M. Bove and Dr B. Aubert about the project activities. They had suggested to revise some of the activities. Accordingly one of the activities "Study the possibility of transoverial transmission of greening by *D.citri*" has been cancelled because it was confirmed that Capoor (1967) had already studied about this and determined that it was not possible. On the other hand "Phytopathological survey on greening incidence of different citrus orchards was added. It is essential to asses the effectiveness of biological control. It was also pointed out that more experiments should be carried out to find out and confirm local natural enemies- predators and parasites and hyperparasites. In connection with the host plant study, more emphasis should be given to the species of Rutaceae family. Trial with the *Nasturtium tropaelum* plant which is possible antifident plant for vector and can be used for ecological control also has been included as one of the activities of this project. Accordingly revised research activities of this project are as follows.

1. Identification of host plants of *D.citri*.
2. Identification of citrus varietal succeptability to greening vector.
3. Determination of population dynamics and life cycle of *D.citri* in Pokhara valley.
4. Identification of natural enemies of greening vector.
   a)Predators
   b)parasites
5. Trial on integrated citrus protection.
   a) Biological control.
   b) Ecological control
6. Determination of greening incidence and distribution pattern in Pokhara valley.

These changes have been incorporated in the First Sixmonthly Report as well as in the Report of Study visit to Reunion and research activities were conducted according to these changes.

In the following chapter each research activities have been described briefly with their relevance of study and methodology of experiment followed by their results and discussions.

4. DETAILS OF RESEARCH ACTIVITIES AND RESULTS

4.1. Identification of host plants of *D.citri*

D.citri can feed and breed on different species of plants. It is most important to get the knowledge of range of host plants on which it can be preserved as even a single individual of *D.citri* is capable to transmit the disease (Capoor et al 1967). Aubert has reported some host plants of *D.citri* from Reunion but this is the first study in this aspect in Nepal.

This study was conducted at Horticulture Research Station, Pokhara. All the citrus and weeds (except Graminaceae family) were examined. Taxonomic identifcation of plants were done in the Botanical Survey and Herbarium Section, Department of Medicinal Plants, Godavari.

The seedlings of trial plants were grown in earthen pots inside screen house before releasing the vector. The vectors were multiplied on *Murraya paniculata* plants in the seperate chamber of screen house. Trial plants were grouped randomly. Alltogether 7 groups of citrus and other species of Rutaceae family were made. All other weeds were grouped seperatly having 8-10 species. Twigs of *Murraya paniculata* with iv and v instar nymphs were released to each pot plant. Alltogether 200-250 nymphs were released to each group by distributing equally to each plant and kept for 2 months. Experiments were conducted during April to July, 1987. Observations were made weekly to record the condition of nymphs, adults and development of next generation.

D.citri prefers all the citrus species/cultivars on which it feeds, lays eggs and developes nymphal stages (Table 2). The first preferencial plants are *Citrus lemon*, *C.aurantifolia* and *Murraya paniculata* plants. It is explained by the frequent flushing nature of these plants. Second preferencial hosts are *C.reticulata*, *C.sinensis*, *C.volkamariana* and Rangpur lime. All other citrus and *Poncirus trifoliata* serve as third preferencial hosts on which the vector feeds, lays eggs and developes nymphal stages. These results confirm the findings of Aubert (1986) on 13 species of citrus and provide additional informatin on them.

Table 2

List of Host Plants of *D.citri* by type of hosting

S No.	Family	Species/Cultivars	Type of hosting: Leaf Sucking	Egg Laying	Nymphal Development
1.	Rutaceae	*Citrus limon* (L.) Burn	+++	+++	+++
2.	"	*C. aurantifolea* Swingle	+++	+++	+++
3.	"	*C. reticulata* Bl.	++	++	++
4.	"	*C. sinensis* L.	++	++	++
5.	"	*C. volkamariana*	++	++	++
6.	"	Rangpur lime	++	++	++
7.	"	*C. marima* (Burm)	+	+	+
8.	"	*C. pectinifera*	+	+	+
9.	"	*C. micropiera*	+	+	+
10.	"	*C. aurantium* L.	+	+	+
11.	"	*C. macrophylla*	+	+	+
12.	"	*C. limettoides* Tanaka	+	+	+

13.	"	*C. samberi*	+	+	+
14.	"	Few trells early	+	+	+
15.	"	*C. karna*	+	+	+
16.	"	*Troyer citrange*	+	+	+
17.	"	*Kinnow mandarin*	+	+	+
18.	"	*Poncirus trifoliata* L.	+	+	+
19.	"	*Severnia toxifolia*	+	+	+
20.	"	*Murraya paniculate*	+++	+++	+++
21.	"	*M. koenigii*	+	-	-
22.	"	*Aegal marmelos*	+	-	-
23.	Acanthaceae	*Justica procumpens* L.	-	-	-
24.	Amaranthaceae	*Amaranthus blitum* (Minor)	-	-	-
25.	Compositeae	*Ageratum conyzoides* L.	-	-	-
26.	"	*Bidens pilossa* L	-	-	-
27.	"	*Crassocephalum crepidoides* (Benth)	-	-	-
28.	"	*Emilia sonchifolia* (L) DC	-	-	-
29.	"	*Elephantopus scaber* L.	-	-	-
30.	"	*Galisoga ciliata* (Rafin) Blake	-	-	-
31.	"	*Spilanthes acmella* (L) Murr	-	-	-
32.	"	*Synedrella nodiflora* Gurth	-	-	-
33.	Convolvulaceae	*Ipomoea* sp.	-	-	-
34.	Euphorbiacae	*Chamaesyce hirta* (L) Millsp	-	-	-
35.	Gesneriaceae	*Rhynchoglossum obliquum* Blume	-	-	-
36.	Labiatae	*Chenopodium umbrosum* (M. Bieb) C. Koch	-	-	-
37.	"	*Leucas lavandulaefolia* J. E. Smith	-	-	-
38.	"	*Scutellaria repens* Buch. - Ham. ex D. Don	-	-	-
39.	"	*Dysophylla auricularia* (L) Blume	-	-	-
40	Leguminosae	*Desmodium heterocarpon* (L) D. C.	-	-	-
41.	"	*Memosa pudica* L.	-	-	-
42.	Malvaceae	*Sida acuta* Burm.	-	-	-
43.	Polygonaceae	*Persicaria nepalensis* (Meisn) H. Gross	-	-	-
44.	"	*Persicaria posumbo* (D. Don) H. Gross	-	-	-
45.	Rubiaceae	*Borreria latifolia* Schumann	-	-	-
46.	Solanaceae	*Solanum nigrum* L.	-	-	-
47.	Tiliaceae	*Triumfetta bartramia* L.	-	-	-
48.	Urticaceae	*Boehmeria platyphylla* D.Don	-	-	-
49.	"	*Gonostegia hirta* (Blume) Miq	-	-	-

Among other species of Rutaceae family, *Murraya panaculata* is the most favourable host plant. *Murraya koenigii* and *Aegal marmelos* serve as the alternate host plants.

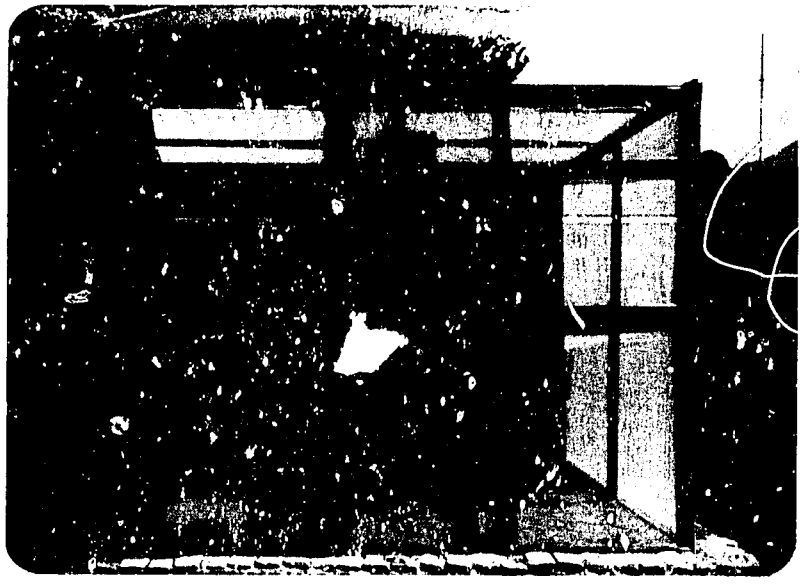


Photo 1. General View of Screen House

Photo 2. Trial plants inside the Screen House

D.citri sucks their leaves without laying eggs and development of nymphal stages. Regarding the weeds, not a single species under our study was found to be a host plant of *D.citri*. All the nymphs released to these plants either died or migrated to citrus seedlings grown near by.

Above mentioned facts suggest that *D.citri* is very specific in nature and the range of host plants are confined to the species of Rutaceae family only. Weeds of other families don't preseve the vector, hence one should not be worried about the weeds regarding the control of greening vector. *M. paniculata*, *M. koenigii* and *Aegal marmelos* should be considered along with other relatives of citrus in developing and applying any controlling measures against vector. They should be included in the management of biological control of vector by using its parasite *T.radiatus*. Effective control of the vector will lead to the successful control of CGD.

4.2 Identification of Citrus varietal Succeptability to *D.citri*

This study was conducted to find out the resistant and succeptable citrus varieties/cultivars to greening vector. It is needed to explore the possibility of ecological control of CGD.

All the citrus species/cultivars grown at the Horticulture Research station, Pokhara, as well as mandarins from different districts were tested. Altogether 25 species/cultivars of citrus served as the materials for experiment. Seedlings of testing plants were grown in earthen pots under screen house condition. 10- 15 adult psyllids previously harvourd on *Murraya paniculata* plants were released to each of these seedlings. Observations were conducted in 12 hours intervals for one week to record their mortality.

All the citrus species/cultivars under our study were found succeptable to *D.citri* as all the released individuals were found alive and feeding on each of the tested plants (table 3). Our field observations and previous studies also confirmed this fact. *D.citri* can feed and breed on most of the citrus species except *Severnia horifolia* on which the greening vector can feed only without laying eggs and developing nymphal stages.

Table 3

Citrus Varietal Succeptability to *D.citri*

Species	Variety/ culivar	Degree of succeptability
Citrus reticulata (Suntala)	Pokhara local	II
	Gorakha local	II
	Dhankutta local	II
	Lamjung local	II
	Sailyan local	II
	Syanja local	I
	Kinnow	I
	Fewtrells early	II
Citrus lemon	Eureka lemon	I
	Pokhara local	I
Citrus aurentifolia	Kagzi lime	I

Citrus sinensis	Sindhuli local	I
	Satgudhi local	I
	Malta common	I
	Valencia late	I
	Mosaumbi	I
Citrus maxima		II
Citrus pectinifera		II
Citrus microptera		III
Citrus aurantium		II
Citrus limetoides		III
Citrus rambhiri		III
Citrus karnakhatta		III
Poncirus trifoliata		III
Severnia boxifolia		

Kapoor et al (1967) had examined 71 citrus species/cultivars for their succeptability to the infestation of citrus psylla and the incidence of greening disease. He catagorised them as resistant, commercially resistant, mildly succeptable, moderately succeptable and higly succeptable. They also reported that citrus species/cultivars which were either resistant or commercially resistant to the citrus psylla were free from greening infection, where as those species/cultivars which were mildly to highly succeptable to psyllid were showing a very high incidence of CGD. However our findings clearly show the succeptability of all citrus species/cultivars but may be catagorised as I, II, and III preferential host plants which is directly related with the flushing rythms rather than genotype of citrus species. It is therefore can be concluded that all the citrus under our study were found to be succeptable with different degree of succeptability and they don't have significant role for ecological control of greening vector.

4.3 Determination of population dynamics and life cycle of greening vector in Pokhara valley

This study was envisaged to find out the dynamics of population of *D.citri* (Kuw) which is essential to workout the calendar of operation for its control. *D.citri* was reported from Pokhara Valley during mid sixties (Rana and Sharma 1964, Catling 1967) but detail study of this vector was not conducted in our country.

Population dynamics of the greening vector was studied in 10 randomly selected Eureka Lemon trees at the Horticulture Research Station, Pokhara. Ten twigs with the highest populations from four geographical directions of each tree at different levels were recorded and all the stages of *D.citri* were counted with the aid of a hand lense for eggs. Observations were carried out weekly during 1980 to 1985. Meteorological data were also recorded in parallel fashion.

The population of *D.citri* reaches at its maximum of 2500 eggs, 828 nymphs and 188 adults per 10 twigs during May, June and July before monsoon during very hot, dry weather, minimums were 120 eggs, 20 nymphs and 1 adult per 10 twigs in winter (Fig. 1, 2). The peaks of population of eggs and nymphs indicate the number of generations of *D.citri* a year.

D.citri prefers the lower altitude up to 1200 m above sea level where hot and dry conditions exist (Bove 1986, Aubert 1986). Coordinates of Horticulture Research

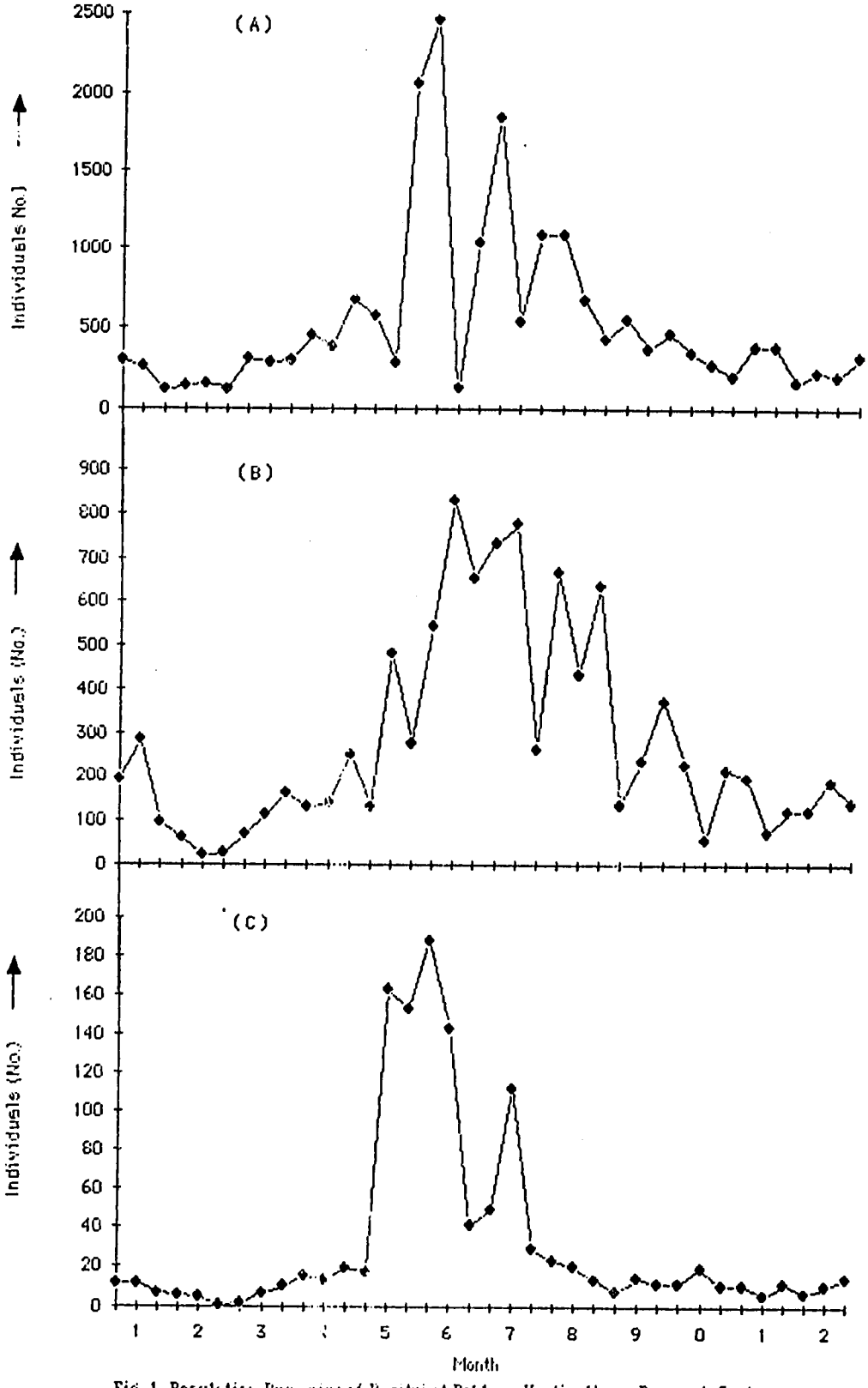


Fig. 1. Population Dynamics of B. citri at Pokhara Horticulture Research Centre
Average Number/10 leaves; A- Eggs, B- Nymphs, C- Adults.

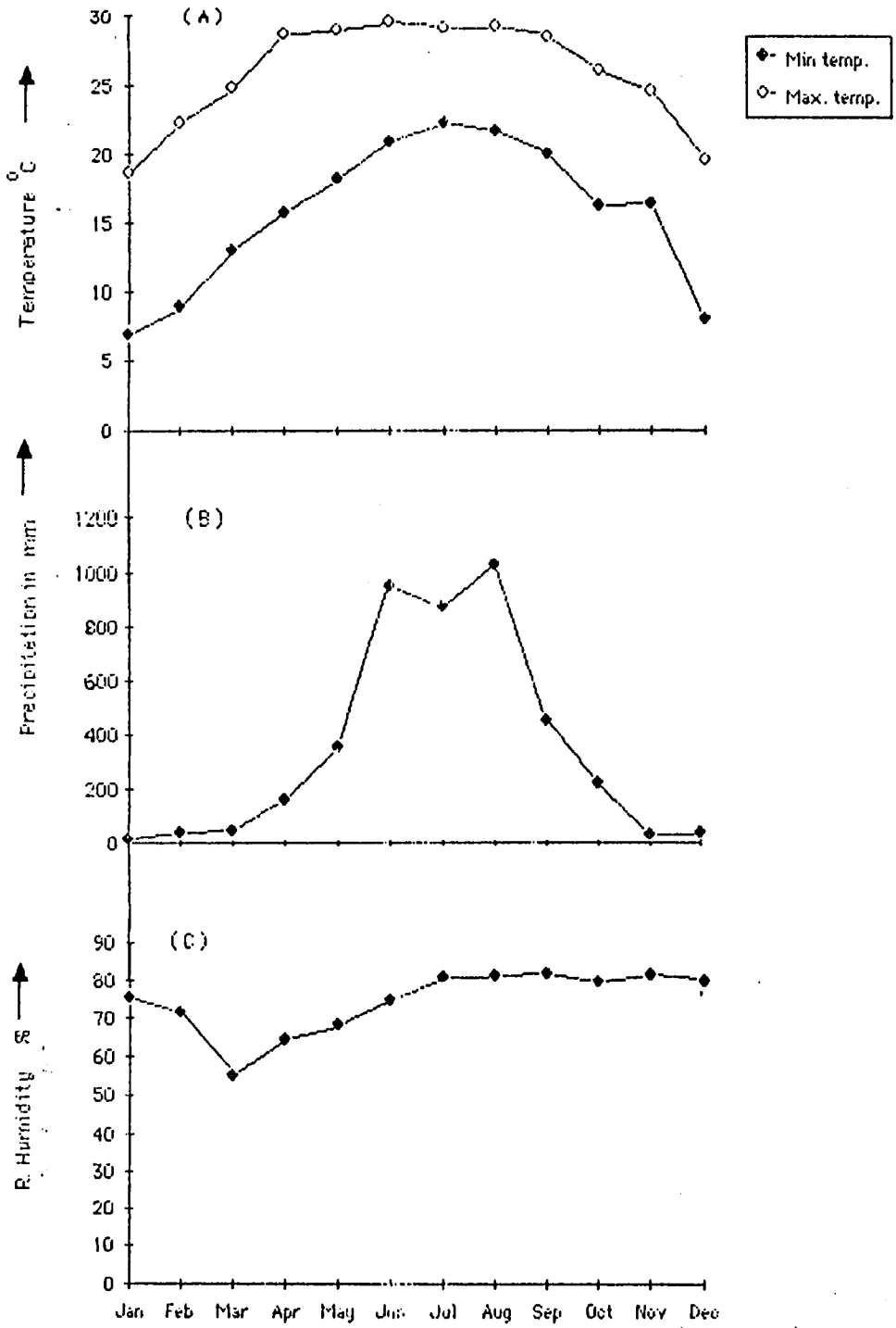


Fig. 2. Meteorological data of Pokhara Valley, 5 years average (1980 - 1985)
A= Average Temperature, B= Precipitation, C= Relative humidity

Station, Pokhara are longitude 83. 590 E, latitude 28. 150 N, and altitude 935 m above sea level. Rainy season starts in the middle of June and ends by the end of October. Usually there is little rainfall in winter. All these factors provide favourable condition for *D.citri* during summer and therefore, population is very high.

During rainy season i.e. in July - October the populations of adults decrease, although there is a comparatively high population of eggs and nymphs. The authors have observed that heavy rain fall eliminates the eggs and nymphs whereas the adults can hide on the lower surface of the leaves and twigs of inner-lower portion of the tree. It is clear that any measures for the control of *D.citri* should be undertaken mainly at the end of the rainy season.

Since greening can be transmitted either with the help of vector or by bud, it is obvious that only the vector *D. citri* is responsible to spread the infection from diseased plants to healthy ones in the light of seedling cultivation in Nepal. Therefore, measures have to be taken to control the vector to overcome the CGD in Pokhara.

4.4. Identification of Natural Enemies of *D. citri*

This study was conducted to find out the frequently occuring natural enemies - predators and parasites which could be applied for biological control against *D.citri* This study was divided into two parts; study of predators and study of parasites.

4.4.1 Study of predators

The main objective of this study is to assess the presence of predators of *D.citri* in Pokhara valley.

All the common predators of coccids, psyllids and aphids were collected from the mandarin orchard of Horticulture Research Station, Pokhara by conducting regular weekly survey during 1985-1986. Taxonomic identification of insects was conducted at Entomology Division, Department of Agriculture, Nepal. Their predating characteristics were determined on the basis of visual observations and by reviewing the available literature on predators of *D. citri*

The following species of predators belonging to three families of Coleoptera were recorded during the survey at mandarin orchard of Horticulture Research Station, Pokhara.

Table- 4

Species	Family
1. *Chilochorus nigritus* F.	Coccinelidae
2. *Coccinella 7-punctata* L.	"
3. *Coelophora nepalensis* Cr.	"
4. *Jauravia quadrinotata* Kapur	"
5. *Leis 15-maculata* Hope	"
6. *Menochilus sexmaculatus* F.	"
7. *Nodostoma* spp.	Eumolpidae
8. *Allographa* spp	Sirphydae

These predators prey on immobile or slow moving insects such as aphids, scales and nymphs of psyllids. Out of these predators *Chilochorus nigritus* F. was found as one of the effective predators of *D. citri* Our observations confirmed the findings of other authors (Catling 1970, Aubert 1986). It attacks mostly II, III and IV instar nymphs of

Photo 3. Predator Chilochorus nigritus

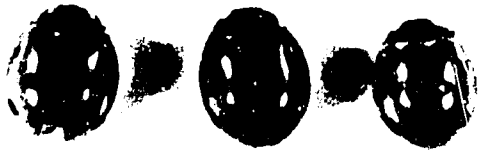


Photo 4. Predator Coccinnella 7- punctata

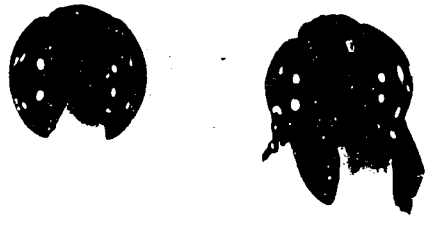


Photo 5. Predator Leis 15- maculata Hope

D.citri. It is note worthy that use of this predator for biological control of greening vector has its limitations due to its lack of specificity in predating the nymphs of *D.citri*, however it can be included in the complex of natural enemies for biological control against greening vector.

4.4.2. Study of Parasites

This study was carried out to reveal the presence of parasites of *D.citri* in Pokhara. After the determination of absence of parasites of *D.citri* in Pokhara, a survey was conducted in other districts of the country to reveal the distribution of *D.citri* as well as its parasites.

Citrus orchards as well as other Rutacious plants were examined thorougly for the presence of psyllid vectors by collecting adults with a mouth aspirator, following a technique described by Aubert (1985). Survey was conducted in Morang, Dhankuta, Saptari, Dhanusa, Siraha, Mahotari, Sarlahi, Chitwan, Kathmandu, Dhading, Gorkha, Lamjung, Tanhun, Kaski, Syanja, Dang and Sallyan districts during the Spring flush 1986. At the same time nymphs of *D.citri* were also examined for the presence of parasites with the help of hand lense to identify the symptoms of parasitism. In case of parasitised nymphs the material was collected and put into hatching boxes. After 2-3 days of incubation hatched parasites and symptoms of parasitism on nymphs were examined under light microscope. To confirm the identity of parasites some individuals were dispatched to known taxonomists of British Museum of Natural History (BMNH) through Dr. Aubert, IRFA, Reunion, France.

T. radiatus was obtained from Morang and Siraha districts only during our study. There was a clear symptoms of parasitism on *D. citri* nymphs of III, IV and V instar nymphs with typical exit hole on the thorax of nymphs exoskeleton. Likewise few nymphs with typical symptoms of parasitsm of *Diaphorencirtus alegharensis* were also observed in those areas. Adult individuals of these parasites were easily identified on the basis of morphological characteristics which were also confirmed by the taxonomists of BMNH. No hyper parasites were found in all the samples studied.
T. radiatus and *D. alegharensis* were limited only in Terai belt although *D. citri* was distributed in mountain and valleys. It can be explained by the fact that *D. citri* was recently introduced to these areas with out parasites. It is positive indication for the application of biological control against greening vector in these areas, because there is no chance of existence of hyper parasites which could limit the efffect of parasites, provided the parasites are aclimatised.

The survey revealed that *D. citri* was recorded (Fig. 3) in abundant number (20-25 adults/5 minutes aspiration) on Citrus and *M. paniculata* host plants in Terai districts ; Morang, Sunsari, Saptari, Siraha, Dhanusa, Mahotari, Sarlahi, Chitwan and Dang valley. It is explained by the fact that altitude of these areas ranges from 100- 700 m above sea level and hot and dry climate of these districts is most suitable for *D. citri*. The average number recorded in Pokhara reached 8-10 adults/5 minutes aspiration, while the number of *D. citri* was very low (not more than 5 adults/5 minutes aspiration) in some pocket areas of Kathmandu valley (1350 m of elevation). It shows the adoptation of the insect in cooler areas. *D. citri* was not found in Dhankuta, Gorkha and Lamjung districts nor in Sallyan during our study. But there is a chance of spreading the vector in these areas from near by districts if the internal quarantine is not properly maintained.

NEPAL

(SCALE-1:2000000)

Distribution of Vector and Parasite

INDEX

International, Regional and district boundary

** Presence of Vector and Parasite

++ Presence of Vector only

oo Absence of both

Districts Surveyed

1. Morang 2. Dhankutta 3. Sunsari 4. Saptari 5. Siraha 6. Dhanusha 7. Mahotari 8. Sarlahi 9. Chitwan 10. Kathmandu 11. Dhading 12. Gorkha 13. Lamjung 14. Tanahu 15. Kaski 16. Syanja 17. Dang 18. Salyan

4.5. Integrated citrus protection

One of the main objectives of this project was to develop biological and ecological control of greening vector. On the basis of above described studies and review of available literature, we selected exoparasite *T. radiatus* for biological control and supposed antifident plant *Nasturtium tropaelum* for ecological control of greening vector and accordingly the studies were conducted on these aspects.

4.5.1. Biological Control of Greening Vector

Effectiveness of *T. radiatus* was first evaluated under screen house condition. After getting promising results from this trial, effectiveness of the parasite *T. radiatus* were confirmed in field conditions.

Pot cultured citrus and Murraya seedling plants were grown in screen house. More than 50 V instar nymphs of *D. citri* were released to these plants and cultured for 2 months. Only 10 adults of *T. radiatus* newly hatched, from the nymphs of citrus psylla collected from Tarai were released on the above mentioned culture of *D. citri*. All the normal and parasitised nymphs of the vector were observed on the hosts and counted seperately after one month of their release.

Field trials on biological control were conducted in Kinnow orchard of Horticulture Research Station, Pokhara. Adult parasites in a number of 15-20 were released to each [illegible]ted 4 trees during early spring, 1987. Population of *D. citri* was recorded before and after release of parasites by counting the number of eggs, nymphs and adults on randomly selected 16 twigs of these 4 trees. Likewise 15-20 individuals of parasites were released in the farmers orchards of Hyanja and Bhalam this year.

Among the 10 adults of *T. radiatus*, 4 individuals died within 3 days and there were left only 6 individuals alive. The experiment was conducted in summer 1986, under favourable hot & dry condition for the multiplication of vector *D. citri* as well as parasite. Vectors laid eggs in a good number and a lot of nymphs were emerged. At the same time parasite *T. radiatus* also got sufficient substract for egg laying which resulted in increased number of parasitised nymphs. The results of the experiment on biological control under screen house conditions seemed to be encouraging (Table 5) as the percentage of parasitism exceeds 90% in some cases.

Table-5

Biological Control Of Psyllid Vector by *T. radiatus* in Pokhara

S. No.	Cultivars	No. of *D. citri* nymphs with Parasitism	No. of *D. citri* nymphs without Parasitism	% of Parasitism (%)
1.	*Citrus sinensis*	205	10	95. 3
2.	*C. reticulata*	60	5	91. 3
3.	*C. limon*	15	4	78. 9
4.	*C. aurantifolia*	12	3	80. 0
5.	*Poncirus trifoliata*	6	3	66. 6
6.	*Murraya paniculata*	2	3	40. 0

The difference in the number of nymphs in different species is explained by the fact that plants of *Citrus sinensis* were vegetatively well developed and those of *Murraya* were very tender. Therefore it does not contradict the findings of Aubert (1985) that *Murraya paniculata* and *Citrus aurantifolia* are preferential and other species included in experiment are simply common host plants and *Poncirus trifoliata* is occassional one. Therefore in the case of parasite also and the percentage of parasitism is higher in one host plant than the other.

T. radiatus was not yet recorded in Pokhara valley (9). Therefore, it could be safely considered that the parasites of *T. radiatus* which can counter balance the population of primary parasite. This fact and the encouraging result of biological control against *D. citri* in screen house condition, enable us to conclude that introduced *T. radiatus* from Terai and reared in screen house will be effective for the biological control against greening vector *D. citri* at least in the context of Pokhara Valley. Therfore biological control of *D. citri* in the farmers field will be carried out in near future in Pokhara and its vicinity where the *Citrus psylla* is prevalent.

Population of *D. citri* before releasing the parasites were recorded on April, 1987 while the after release were done on April 1988, in the field. Results (table 5) show 50% reduction of adults in after release records as compared to that of pre release population. Ettiene etal (1980) obtained 60- 65 % reduction of *D. citri* population in the similar study in Reunion.

Table 6

Population of *D. citri* in Kinnow Orchad before and after releasing *T. radiatus*

Items	Number of individuals/ 10 twgs		
	Eggs	Nymphs	Adults
Before releasing the parasite	43	88	6
After releasing the parasite	26	39	3
Percentage of decrease in population	60	44	50

There was less reduction of greening vector population in our study which can be explained by the fact that there exist comparatively low population of vector in this orchard because several citrus trees, standing near by were cut down.

Parasites were observed after one year and were quite aggressive to parasitise the nymphs of citrus psylla. This fact suggests that *T. radiatus* has been aclimatised in the field conditions of Pokhara Valley and can be done so in similar conditions. These facts along with the results obtained from the experiment conducted in screen house conditions enable us to confirm the prospective of biological control against *D. citri* in the field conditions of Pokhara Valley where any hyper parasites does not exist.

Photo 6. Parasite Tetrastichus radiatus Waterstone

Photo 7. Parasitised nymph of D.citri
Typical exit hole on the thorax is seen

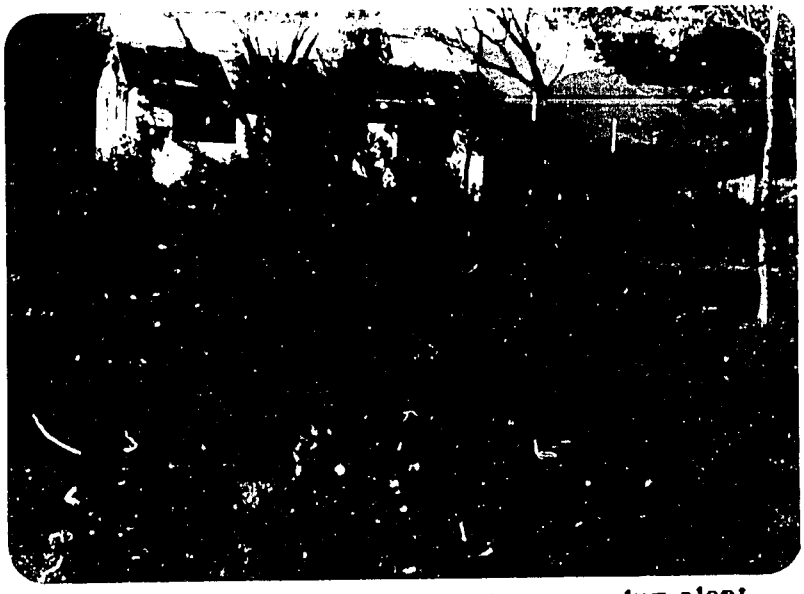


Photo 8. Experiment with Nasturtium tropaelum plant

Photo 9. General View of greening infected mandarin orchard

4.5.2. Study on Ecological Control of Greening Vector

This study was conducted to determine the effect of *Nasturtium tropaelum* plant - supposed antifident of citrus psylla.

Nasturtium seeds were grown in poly bag on February 1987. After one month they were transplanted around the selected 5 kinnow mandarin trees and 5 kinnow mandarin trees were also selected as control. Population of greening vector were recorded in 15 days interval for two months by counting the individuals on randomly selected 5 twigs of each trees.

Population of vector was found comparatively low on kinnow trees associated with flowering *Nasturtium tropaelum* plant (table 7).

Table 7

Population Dynamics of *D. citri* associated with *Nasturtium tropaelum* Plant

Date	Number of adults/5 twigs		Remarks
	With Nasturtium	With out Nasturtium	
20 March,1988	0	4	
5 April, 1988	6	6	
20 April, 1988	4	5	
5 May, 1988	5	15	

There appeared comparatively non significant reduction of population before this stage. It may be due to the very big gap (2-3 m) between the heights of Kinnow and Nasturtium plants. However this result suggests the existence of antifident property of Nasturtium plant with 20-60 % of effectiveness for ecological control of greening vector. Hence it is suggested that study should be conducted on chemical composition of Nasturtium to findout the antifident component which could be extracted. This extract may be used for foliar application on the citrus trees to reduce the population of citrus psylla.

4.6. Greening incidence and distribution pattern in Pokhara Valley

Thrower (1967) mentioned citrus decline first appeared in 1964 in Pokhara valley. Later Catling (1968) visited the area around Pokhara and on the symptomatological grounds considered the decline to be due to greening. Knorr et al (1970,1971) confirmed it to be greening by applying Schwarz's Thin Layer Chromatographic test. They also suggested that the disease was introduced from Saharanpur, India. They also conducted survey on incidence of greening in different citrus orchards of the country and suggested that 54% of the trees in the country were infected with greening. The infection rate in Western Nepal (Pokhara) was 53%, in the Kathmandu Valley it was 43% and in Eastern Nepal 59%. Regmi (1982) also had made similar survey on CGD and reported that the rate of infection had been increased up to 60 - 100% in Pokhara valley, and reduced upto 17-39% in Dhankutta, 25% in Saliyan and only 9. 7% in Sanothimi. Percentage of infected trees was decreased in Dhankuta as

Table 8

Status of greening incidence in different citrus orchards of Pokhara Valley

S.N.	Location of citrus orchard	No. of trees surveyed	No. of infected trees	% of infected trees	% of trees giving +ve reaction on TLC test	No. of trees with the degree of infection			Intensity of infection on diseased plants
						Severe	Mild	Slight	
1.	Batulechour	34	16	47.1	40	15	1	0	96. 8
2.	Begnas	97	16	16.7	27	9	4	3	79. 7
3.	Bhalam	41	20	48.8	21.7	5	9	6	66. 3
4.	Bharat Pokhari	100	0	0.0	0.0	0	0	0	0. 0
5.	Hyanja A	31	5	16.1	27.5	1	2	2	70. 0
6.	Hyanja B	36	0	0.0	0.0	0	0	0	0. 0
7.	Hort. Res. Station A (Local mandarin)	104	104	100.0	100.0	99	5	0	97. 6
8.	Hort. Res. Station B (Kinnow mandarin)	30	30	100.0	100.0	24	6	0	84. 0
		473	191	40.4	39.5	153	27	11	61. 8

many of the infected trees were dug out after the first survey done by Knorr etal. All these data are based on the samples from certain locations. Detail studies on the incidence of CGD in Pokhara Valley are lacking. It is therefore, this study was carriedout to reveal the present situation of CGD incidence in Pokhara Valley.

Properly managed eight citrus (Suntala) orchards of 5 locations of Pokhara Valley were selected for this study. Each trees of these orchards were examined on the basis of visual symptoms. Degree of greening infection was recorded by canopy rating as per the following scale.

Grade 0 - No greening symptoms.
Grade 1 - Greening symptoms on 25% of leaves and twigs.
Grade 2 - Greening symptoms on 26- 50 % of leaves and twigs.
Grade 3 - Greening symptoms on 51-75% of twigs.
Grade 4 - Greening symptoms on more than 75% twigs showing die back.

Trees showing 1 grade of effect were considered as slightly damaged and showing 2-3 were considered as mildly damaged while grade 4 were taken as severly damaged. Phytopathological maps have been prepared on the basis of these data. Thin Layer Chromatographic tests were also conducted to confirm these visual symptoms of CGD.

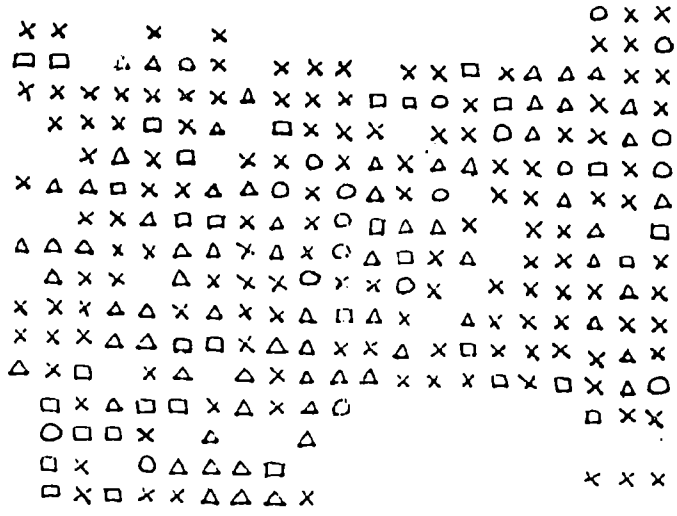
Survey of greening incidence in different citrus orchards of Pokhara valley revealed that the disease has spread in 6 orchards out of 8 surveyed (table 8) Percentage of infected trees varies from 16- 100%. Highest percentage of infected trees was found at the Horticulture Research Station, Malepatan, Pokhara which is due to the cumulative accumulation of infection and the presence of sufficient number of vector. Comparatively high percentage of infected trees (47-49%) at Batulechaur and Bhalam area was observed as most of the plants were originated from the Horticulture Research Station, Malepatan. Low percentage of infected trees were observed at Begnas and Hyanja area where there were comparatively young plantations and effective plant protection measures were conducted in a systemic way which avoided the growth of vector population and consequently limited the secondary infection. Only two orchards did not show any visual symptoms of greening. It may be explained by the fact that most of the trees were comparatively younger and were originated from local seedlings. However there is a chance of secondary infection by vector from near by infected orchards. The intensity of infection on diseased trees was high in all the infected orchards varying from 66 to 98%.

Canopy rating conducted in 1981 shows the distribution pattern of the disease in the orchad - Mandarin Block A of Horticulture Research Station, Pokhara (Fig 5). At that time there were few appearently healthy trees where as the studies conducted in 1986 revealed that all the trees were severely damaged and are not economically useful. The overall progression of the disease was more than 12% of newly severely damaged trees per year in the orchard. Since greening can be transmitted either by vector or by infected bud, it is obvious that only the vector *D. citri* was responsible to spread the disease in this orchad during this period because all these plants were of seedling origin.

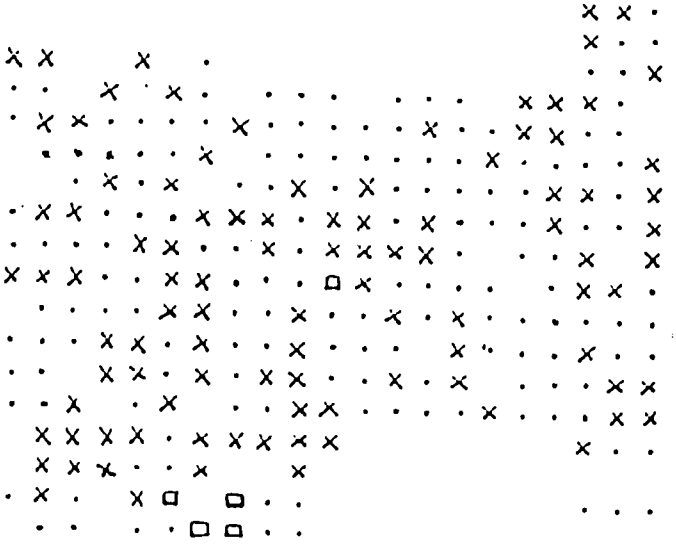
Phytopathological maps of other citrus orchards (Fig 5-11) show the present situation of greening incidence in those orchards.

No detail studies have done to asses the economic loss caused by CGD in Nepal. Regmi(1982) reported that greening infected trees gave 5 times less yield than the

PHYTOPATHOLOGICAL MAP OF GREENING AFFECTED ORCHARD MANDARIN



CONDITION IN 1981



CONDITION IN 1986

FIG.4. BLOCK "A" HORTICULTURE RESEARCH STATION, POKHARA

INDEX - × SEVERE ◻ MILD • NEWLY PLANTED

△ SLIGHT ○ HEALTHY

PHYTOPATHOLOGICAL MAP OF GREENING AFFECTED ORCHARD MANDARIN, HYANJA

FIG.5 HYANJA A

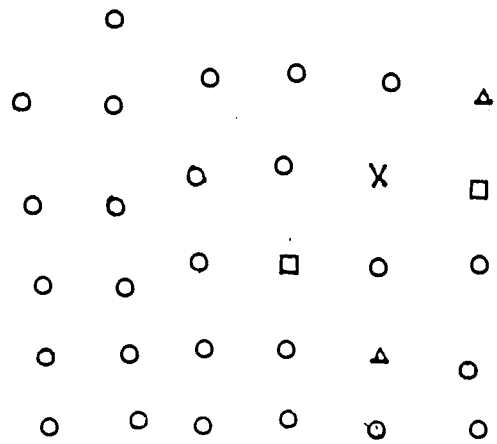
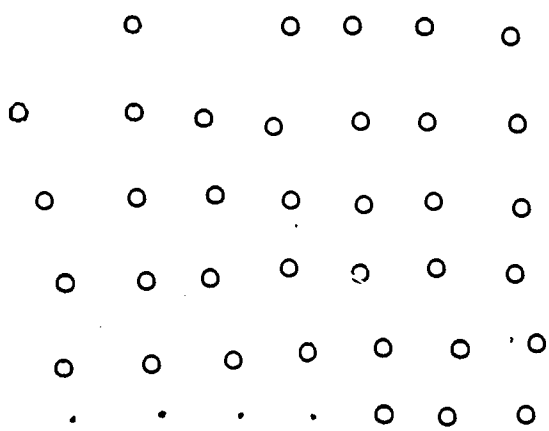


FIG. 6. HYANJA B



O HEALTHY

△ SLIGHT

□ MEDIUM

X SEVERE

PHYTOPATHOLOGICAL MAP OF GREENING AFFECTED ORCHARD

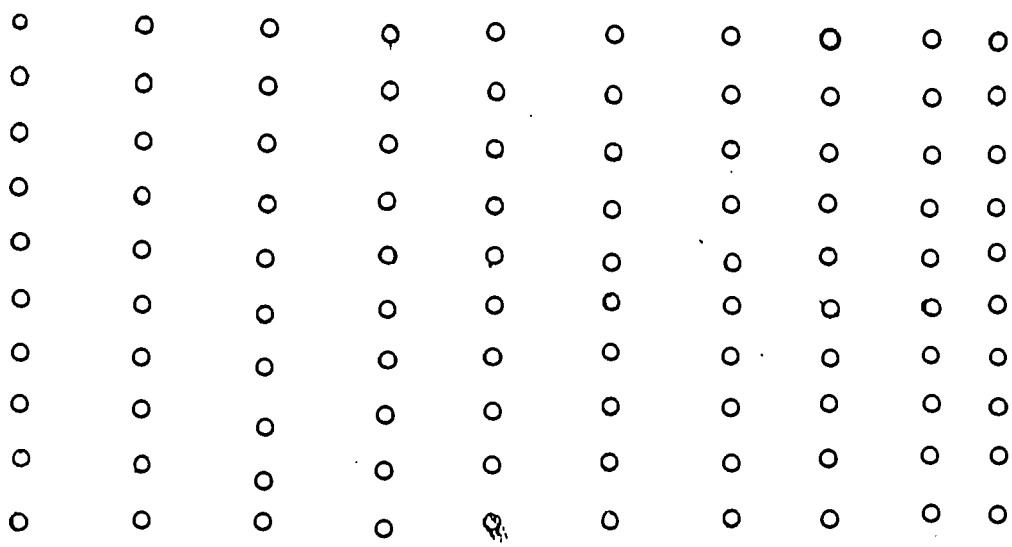


FIG. 7 : BHARAT POKHARI MANDARIN

INDEX -

O HEALTHY

△ SLIGHT

□ MEDIUM

× SEVERE

PHYTOPATHOLOGICAL MAP OF GREENING AFFECTED ORCHARD

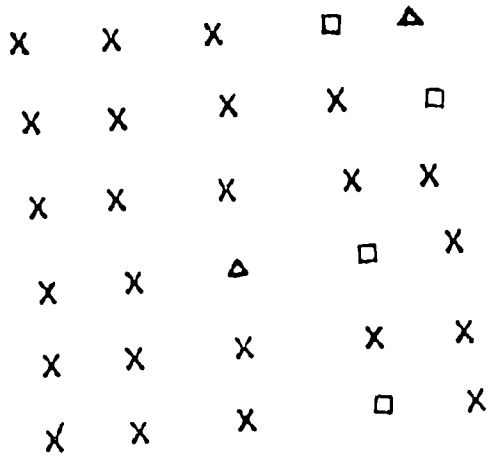


FIG.8. KINNOW ORCHARD HORTICULTURE RESEARCH STATION, POKHARA

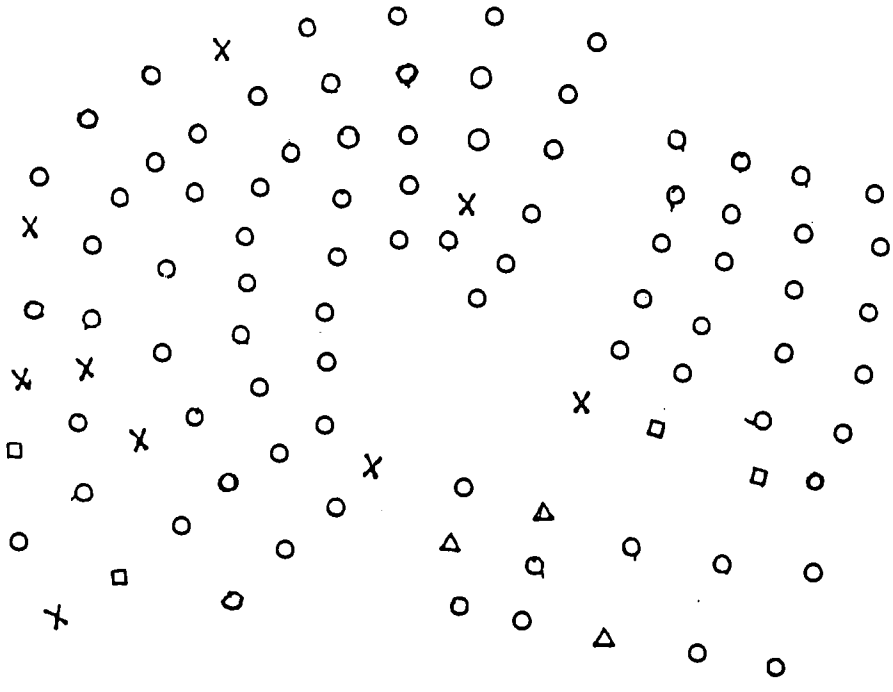


FIG. 9. MANDARIN, BEGNAS

PHYTOPATHOLOGICAL MAP OF GREENING AFFECTED ORCHARD MANDARIN

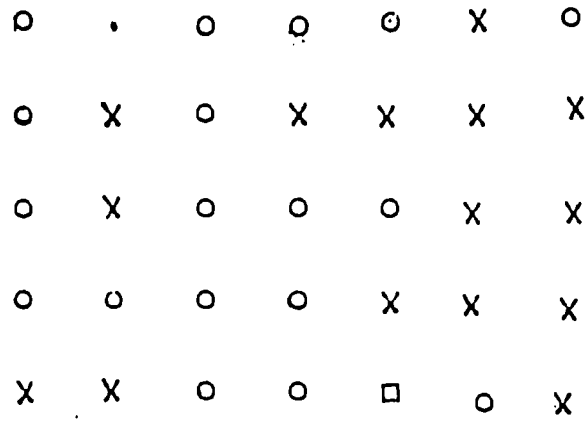


FIG. 10 BATULECHOUR

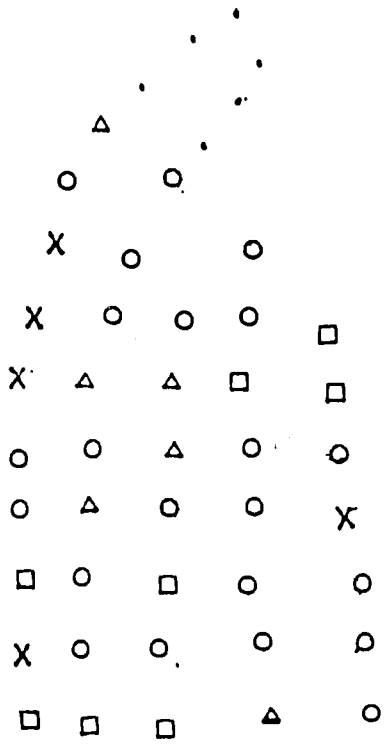


FIG. 11. BHALAM

appearantly healthy ones. There are some informations about the distruction of citrus orchards caused by CGD. During early seventies a campaign was made to destroy all the infected trees to check the spread of greening. Around 15 thousand mandarin trees of farmers orchards were cut down in Pokhara Valley and 15 hundreds of mandarin and orange trees were dug out only in Horticulture Research Station. Replantation of local mandarin was conducted in this Station and in farmers field of this valley during 1972-73. Altogether 12 thousand new plants were supplied to the farmers of this valley to replace the infected orchards. Our findings on incidence of CGD in this valley show that most of them are infected with the disease again. Again more than 700 badly infected citrus trees including those of the Mandarin Block A of Horticulture Research Station, Pokhara, were cut down in 1977. Horticulture Research Station, Pokhara, supplies 3-4 thousand citrus saplings (seedlings, grafted and air layered) to the farmers of priority areas. Looking at the nursery management in the circumstances of abundant greening infection and freely dwelling vector, one can simply assume that most of them must be infected with greening. Therefore it is suggested that production of citrus saplings should be totally stopped or done under green house condition with proper mangement efforts. On the other hand production of citrus saplings by local nurseries to fulfil the local demand on them is most preferable.

5. GENERAL CONCLUSIONS

The following conclusions can be drawn from these studies.

1. Greening disease is a serious problem for citrus production in Pokhara Valley. It is spreading very rapidly in this valley causing severe damage to citrus plants. Primary infection of CGD in nurseries take place with infected budwoods and while secondary infection is done by vector in the field. Therefore both the measures; supply of healthy planting materials and control of vector are equally important to overcome this disease.

2. Range of host plants of greening vector *Diaphorina citri* are limited to the species of Rutaceae family only. Weeds of other families don't serve as host plants of vector. Hence only citrus and some species of Rutaceae family other than citrus should be counted for the management of citrus vector control.

3. All the citrus species/cultivars are succeptable to greening vector, hence ecological control of vector by mixed cropping pattern of different citrus species/ cultivars is not possible.

4. There might be upto 11 generations of *D. citri* in Pokhara valley. The population reaches at it's maximum during May, June and July before monsoon and minimum during winter. Lifecycle of *D. citri* is completed within 20 -25 days during summer and 35-40 days during winter. These facts suggest to conduct any measures for the control of vector before monsoon.

5. There exist 3 species of common predators of citrus psylla, aphids and scales in Pokhara Valley out of which *Chilochorus nigritus* is most effective one to attack the citrus psylla. *D. citri* is dwelling without any parasites in the valleys like Pokhara where as two species of parasites viz *Tetrastichus radiatus* and *Diaphorencirtus elegharensis* are parasiting it in Terai in natural condition.

6. Experiments conducted in screen house condition showed promising results for biological control of greening vector by *T. radiatus*. Field experiments also confirmed these reults. Therefore *T. radiatus* can be used for biological control of greening vector at least in Pokhara valley and in those areas where no hyper parasites are found. Predator *Chilochorus nigritus* can be added in the complex of natural enemies.

7. *Nasturtium tropaelum* plant is less effective to control the greening vector population. It can't be recommended for the ecological control of greening vector.

RECOMMENDATIONS

Considering the feasibility of citrus production in the whole mid hill range of the country and their economic importance. HMG/Nepal has given due priority for citriculture. It has been included as major component in the recently started Hill Fruit Development Project in Eastern Development Region which requires more than two million citrus saplings. Like wise priority areas for citrus cultivation have been selected in 19 districts of other Development Regions which also requires huge number of citrus saplings. In this context supply of healthy planting materials has got a topmost importance as the primary infection of CGD takes place in the nurseries. Secondary infection of greening in the field through vector, so control of vector also has got no less importance. CGD has spread so widely that even the selected mother plants might be infected which require elimination of the infection. On the back ground of these facts and results of our study following recommendations are suggested.

1. Production of citrus saplings should be either totally stopped in the premises of Horticulture Research Station, Pokhara or should be done under well managed green house condition after developing proper indexing facilities and well managed Foundation Stock.

2. It is better to establish local nurseries to fulfil the local demand on citrus saplings in different citrus growing areas under the supervision of plant protection specialists.

3. A plant indicator nursery should be established to index the virus and virus like diseases including CGD at the Horticulture Research Station, Pokhara. Budwoods should be recommended only after indexing on plant indicator or by TLC tests.

4. Research and development work on Production and use of monoclonal antibody and culture of greening organism should be conducted / implemented (already developed by others) quick and reliable diagnostic techniques of greening. This work can be initiated either by RONAST or NCDP but it is better to conduct jointly by them.

5. Internal quarantine should be strictly maintained through out the country to avoid further spread of the greening disease and it's vector.

6. All the greening infected citrus trees should be cut down at the Horticulture Research Station, Pokhara, as they are not economically feasible but serve as the good source of greening infection.

7. Biological control of greening vector by using its parasite *T. radiatus* should be implemented in different citrus orchards of Pokhara valley. Monitoring of greening vector should be initiated in other citrus growing areas of the country too to reveal the possibility of using biological control.

REFERENCES

Altomirano, Gonzales and R.C. Vinas R.C. 1976
Analysis of the Devastation of leaf Mottling (greening) Disease of citrus and its control programme in the Phillipines. In 7th conf. IOCV. Univ. California Press Riverside, pp 22-26

Ariovich D. and H.M. Garnett 1984/1985
Structure of the greening organism. The Citrus and Subtropical Fruit Journal pp. 6-8

Aubert B. 1985
Le greening une maladie infectieuse des agrumes, de origine bacterienne transmise par des Homopteres psyllids contribution a l'etude d'une strategie de lutte applique au cas de lile de la Reunion. Ph. D. Thesis submitted to University of Bordeux II

Aubert. B. 1986.
Trioza erytreae (Delguercio and Diaphorina citri Kuwayama) (Homoptera)
The two citrus psyllidae vector of the greening disease.
Possible Strategies of Control. Monograph. IRFA, Reunion

Aubert B. and Quilici S. 1984
Biological Control of the African and Asian Citrus Psyllids (Homoptera : Psylloidae) through Eulophid and encyrtid parasites (Hymenoptera :Chalcidoidea) in Reunion island. In Proc. 9th conf. IOCV. Univ. California Press Riverside pp. 100-108

Aubert B. and Quilici S. 1986
Results of the monitoring citrus psylla on yellow traps in Reunion island. In Proc. X conf. IOCV underpress.

Bove J.M., Bonnet P., Garnier M and Aubert B. 1980
Pennicillin and Tetracycline treatments of greening disease- affected citrus plants in the glasshouse and the Bacterial nature of the Prokaryote associated with greening. In Proc. 8th conf. IOCV. Univ. California press, Riverside. pp.91-102

Bove, J.M. and Saglio P. 1974
Stubborn and greening: a review, 1969-1972. In Proc 6th conf. IOCV Univ. Calif., Div. Agr. Sci., Richmond, pp. 1-11

Bove J.M. 1986
Greening in the Arabian Peninsula toward new techniques for its detection and control. FAO plant. prot. Bull. Vol.34. N.1. pp.7-14

Broadbent P. 1983
Citrus greening and virus diseases in China and South East Asia
Asia and Pacific Plant Protection Commission.
Technical Document N. 132

Calavan E.C., Stanley M.M. and E.H. McEachern 1978
Registration, certification and indexing of citrus trees. Citrus Industry
Vol. IV, pp. 185-222.

Capoor S.P., D. G. Rao and S. M. Viswanath, 1967.
Diaphorina citri Kuway. A vector of the greening disease of citrus in India. Indian J. of Agri. Sc. 572-576

Catling H.D. ,Garnier M. Bove J.M. 1978
Presence of citrus greening disease in Bangaladesh and a new method for rapid diagnosis. FAO Plant Prot. Bull. Vol. 26, N. !. pp. 16-18

Catling H.D. 1968.
Report of a visit to Nepal. FAO Report Ph. T/67/2 (mimiograph)

Catling, H.D. 1970
Distribution of the psyllid vectors of citrus greening disease with notes on the biology and bionomics of Diaphorina citri Kuw. Plant Prot. Bull. FAO Vol.18, N. 1, pp. 8-15.

Cochran L.C. 1976
The occurence of greening in Pakistan. In Proc. 7th conf. IOCV Univ. California Press, Riverside pp. 21

Consolidated Report, RONAST. 1985.

Daniel N. and J.M. Bove 1984.
The greening organism is a gram negative Bacterium-In Proc 9th conf. IOCV. Univ. California Press Riverside. pp. 109-114

Duncan F and H.M. Garnett 1985
Serological studies of the greening organism. The Citrus and Subtropical Fruit Journal pp. 9-10

Etienne J. and B. Aubert 1980
Biological control of Psyllid vectors of greening Disease on Reunion Island. In Proc. 8th conf. IOCV Univ. California Press, Riverside. pp. 118-122

Fifth Five Year Plan (1985-1990)

Garnett H. M. 1984/1985
Isolation of the greening organism. The citrus and subtropical / Fruit Journal. pp.4-5

Garnier M. and J.M. Bove 1983
Transmission of the organism associated with citrus greening disease from Sweet Orange to Periwinkle by dodder, Phytopathology Vol. 73 N. 10,
pp. 1358-1363

Garnier M. ,G. Martin. Gros and J.M. Bove 1987.
Monoclonal antibodies against the bacterium like organism associated with citrus greening disease. Ann. Inst. Pasteur/Microbial. 138, pp. 639-650

Garnier M., N Daniel and J. M. Bove 1984
Actology of citrus greening disease Ann. Microbial. (Inst. Pasteur) 1984
135 A. pp. 169-171.

Ghosh S. K. ,Raychaudhari S.P.,Varma, A. and Nariani T. K. 1971
Isolation and culture of mycoplasma associated with citrus greening disease Curr. Sci. 11. pp. 299-300.
Hill Fruit Development Project
Final Feasibility Study Report May 1987, APROSC.

Joomaye E. and Aubert B. 1986.
Biological control of Trioza erytreae (Del Guercio) and Diaphorina citri Kuwayama (Homoptera:Psyllidae) in Mauritius Island (X IOCV under Press).

Knorr, L. C. ,Moin Shah S., Gupta, O.P. 1970
Greening disease of citrus in Nepal. Plant disease reporter. Vol. 54, N. 12, pp.1092-1095

Knorr L.C., Moin Shah S. 1971
World citrus problems - V Nepal FAO Plant Prot. Bull.
Vol. 19. N.4 pp. 74-78

Lafleche D.& Bove J. M. 1970
Mycoplasmes dans les agrumes atlients de greening de stubborn on de maladies similaires. Fruits. 25, pp.455-465.

Lafleche D., Bove J. M. 1970
Structure de type mycoplasma dans les foccilles d'orangers atteints de la maladie du greening. CA Acad. Sci Ser D.V. 270, N. 15 pp. 1915-1917

Lama T.K. 1982
Screening of different species/ cultivars of citrus for greening marker substance IAAS J. Vol. 3 N.1 pp. 43-45.

Martin- Gros M., Iskra, M. Garnier J. Gandar and J.M. Bove. 1987
Production of monoclonal antibodies against phloem limited pathogene prokaryotes of plants: a general procedure using extracts from infected periwinkels. Ann. Inst. Pasteur/ Microbial. 138, pp. 625-637.

Martinez A. L., Nora D. M. ,Armedilla A. L. 1970
Suppression of symptoms of citrus greening disease in the Phillipines by treatment with tetracycline antibodies. Plant Dis Report. Vol. 54, N. 12 pp. 1007-1009.

Massonie G., Garnier M and J. M. Bove 1976
Transmission of Indian citrus greening by Trioza erytreae (Del Guircio) The vector of South African greening. In Proc. 7th conf. IOCV
Univ. California Riverside pp. 18-20

Miyakawa T. 1979.
Suppressive effect of penicilline and some other antibodies on symptom development of citrus likubin (greening) Ann. Phytopath. Soc. Japan. Vol. 45, N.3, pp. 401-403

Moll J.N., Martin M.M. 1974
Comparision of the organism causing greening disease with several plant pathogenic gram negative bacteria, rickettsia-like organisms and mycoplasma like organisms, pp. 89-96. In Colloque Inst. Natle, Sante Rech. Med. (INSERM), Bordeux,33.

Moll J.N. and Martin M.M. 1973
Electron microscopy evidence that citrus psylla T. erytreae D.G. (Homoptera Psyllidae) on citrus limon and four indegenous host plants. Phytophylactica sp. pp. 41-44

Moll J. N., Van Vuueren and D.N. Milne 1960
Greening disease The South African Situation. In Proc. 8th conf.IOCV Univ. California Press, Riverside pp. 109-117.

Nariani T. K. & Bhagbati K. N. 1980
Studies on the Therapeutic and Insect Vector Control of the greening disease In Proc. 8th conf. IOCV. Univ. California Press, Riverside pp. 122-128

Navarro L. 1981
Citrus shoot tip grafting in vitro (STG) and its application a review Proc. Int. Soc. Citriculture 452-456

Oberholzer P.C.G., Von Standen D.F.A., Basson W. J. 1965
Greening disease of sweet orange in South Africa. In Proc. 3rd conf. IOCV. Univ.Florida Press, Gainesville pp. 213-219

Rana P.N. and Sharma K. C. 1965
Preliminary list of crop pests in Nepal. Tech. Doc. FAO Pl. Prot Comm. S.E.Asia N. 49 pp-6

Regmi C. 1982.
Mycoplasmalike disease of citrus in Nepal and USSR (spread, effect, aetology, varietal resistance, possible vectors).Ph.D. dissertation. Moscow Agricultural Academy, Moscow. pp. 144

Schwarz R.E.1968.
Thin layer chromatographical studies on phenolic markers of the greening virus in verious citrus species S. Afr. J. Agr. Sci. Vol.II, N. 4 pp. 797-802.

Schneider H. 1968
Anatomy of greening diseased Sweet Orange shoots. Phytopathology Vol. 58, N. 8, pp.1155-1160

Shah S. M. 1984.
Citrus decline problem in Nepal. Natures paradise (ed by T.C. Majupuria) White Lotus Co. Bangkok pp. 335-336.

Shui-Chen Chin and Ching-Chin Chin 1988
Control of Diaphorina citri in Taiwan with imported Tetrastichus radiatus. In 5th newsletter of Intercountry UNDP-FAO Regional Project of Governments of China, Indonesia, Malaysia, Phillipines and Thailand for the control of the Citrus Greening Disease.

Sodhi S.S., Dhillon S.S., Jeyrajan R.& Cheema S.S. 1973
Isolation and characterisation of mycoplasma like organisms with citrus greening diseases. Indian J. Microbial 1973 N.13, pp.13-16.

Su H.J. and Chang S.C. 1976
The responses of the likubin pathogen to Antibiotics and heat therapy in Proc.7th conf. IOCV Univ. California Press Riverside. pp. 27-34.

Su H.J. and Matsumotto T. 1972
Further studies on the complex causing likubin of citrus in Taiwan.In Proc. 5th IOCV conf. univ. Florida press, Gainesville pp. 28-34

Tirtawidjaja S. 1980
Citrus Virus Research in Indonesia
In Proc. 8th conf. IOCV. Univ. Florida Press Gainesville. 129-132.

Van Bruggen A H C and A. Yilma. 1985
Virus & viruslike diseases of citrus in Ethiopia. FAO Plant Prot. Bull Vol. 33
N 1 pp. 2-12

Virus and virus like diseases of citrus. A collection of color slides, IOCV. edtd by J.M.Bove
& R.Vogel. Vol. I & IV Chapter G.reening. 1981.

OTHER ACTIVITIES

Visit/ Participation in scientific institution/ conferences

1. Principle investigator (PI) and co- investigator had 3 weeks study visit to IRFA, Reunion, France on January 1986.

2. PI participated in the X IOCV Conference held on November 1986 in Valencia, Spain.

3. PI participated in Asian Biological Symposium, held on Oct. 1987 in Kualalumpur, Malaysia.

4. PI and co- investigator participated in National Conference on Science & Technology organized by RONAST in Kathmandu on April, 1988.

5. PI partcipatred in PSTC Conference on Biotechnology in health and agriculture organized by Office of the Scientific Advisor USAID, on June 1988 in Wasington D.C, USA.

Publication

1. C. Regmi and T. K. Lama 1988. Greening Incidence and Greening Vector Population in Pokhara. pp. 238-242. Proc. 10 th Conf. IOCV, IOCV Riverside.

2. T.K. Lama, C. Regmi and B. Aubert 1988. Distribution of the Citrus Greening Disease Vector (*Diaphorina citri* Kuw) in Nepal and Attempts to Establish Biological Control. pp. 255-257. Proc. 10 th Conf. IOCV, IOCV Riverside.

3. Regmi. C & T.K.Lama (1987)
   Efforts for the control of citrus greening disease. Vraman. (T.C. Campus), Kathmandu. pp.9-11.

4. Regmi C. & T.K.Lama (1987)
   Natural enemies of citrus greening vector and possibility of their use for biological control. Proc. Asian Biological Symposium, Kualalumpur (under press).

5. Regmi C.& T.K.Lama (1988)
   Range of host plants of *Diaphorina citri* Kuw - a vector of citrus greening disease. Proc. National Conference on Science & Technology, Nepal (under press).