PLANT VIRUS DISEASES OF HORTICULTURAL CROPS IN THE TROPICS AND SUBTROPICS

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FOREWORD

This book is the proceedings of the FFTC seminar 'Virus Research of Horticultural Crops in the Tropics' held at Taiwan Agricultural Research Institute in September 1985. As the range of the seminar was not limited to tropical crops, therefore the book title has been extended to include subtropical fruit crops. Cosponsors at the seminar included the Taiwan Agricultural Research Institute (TARI), the Council of Agriculture (COA) and the Taiwan Provincial Department of Agriculture and Forestry (PADF).

The material in the book has been ordered on a crop basis rather in order of presentation at the seminar. The General Discussion section was particularly relevant to the control of papaya ringspot disease and thus the chapters on papaya ringspot are placed before the General Discussion.

FFTC deeply appreciates the authors' participation at the seminar and their contributions toward this publication. Thanks is also due to Dr. R.J. Chiu for his proof reading of Chinese translations of abstracts. We further thank the cosponsors, whose support in staff and provision of facilities made the seminar possible.

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Director/FFTC

PREFACE

Virus diseases of horticultural crops in the tropics of the Asian and Pacific region are part of a world wide picture in which the plight of citrus, papaya and banana growers affected by various viral attacks on their fruit trees nave received most publicity. There is a large amount of identification work to be done on many crops but it is becoming apparent that viral diseases of the region are for the most part similar to those prevalent throughout the world horticultural crops.

The current situation of rice surplus in some countries of the region is necessitating that farmers diversify their output, turning rice over for horticultural crops. However, where agriculturalists have led the farmers into new crops, it is frequently the case that elated with the establishment of a new cash crop the disease situation has been neglected, until too late. New crops established on clean ground with clean material could have assured a much better future.

A comprehensive list of viral diseases of cucurbits is presented in this book with sources of resistance available, transmission factors and recommendations for control programs. They are a worldwide problem and specific mention is made of zucchini yellow mosaic virus (ZYMV), believed severely damaging to pumpkin crops in Malaysia and the object of much research and breeding programs in Thailand, Taiwan and Japan. So far all sources of resistance to ZYMV in cucurbits have been derived from tropical origins; the task remains to breed these into commercially acceptable varieties.

In solanaceous crops of Malaysia, viruses cause extensive damage to chillies (*Capsicum* spp.). In Thailand three viruses are identified as the main cause of crop damage to chillies, these are cucumber mosaic virus, tobacco mosaic virus and pepper mottle virus found in mixed infection. Tomato crops in Malaysia and Thailand are affected by tomato yellow leaf curl virus (TYLCV), causing a 10-100% disease incidence and extensive crop damage. TYLCV transmitted by the vector whitefly, has been shown to have a wide host range. Futile attempts to control whitefly vector of TYLCV in Thailand have turned research to tolerant and resistant varieties of tomato. It is not mechanically transmissible. Virus purification has been done successfully in Thailand. Extensive work has been done in Taiwan on viral diseases of tomato and Chinese cabbage crops with a view to breeding resistant varieties; plant breeders at the Asian Vegetable Research and Development Center are working on breeding ToMV resistant tomato plants. In Japan the search for an attenuate form of CMV brought to light the strain CMV-SR isolated from a spinach plant, which protected preinoculated tomato plants against the severe CMV strain.

The families convolvulaceae and aroid include important root crops of the region, both sweet potato and taro traditionally having been staples of some communities, and now becoming more prominant as sources of starch and alcohol for industry. In sweet potato in Taiwan three sap transmissible viruses are relevant which often occur and act in complex.

As leaf curl disease they cause significant crop damage. Japan has done considerable work on virus diseases of sweet potato and selected resistant varieties. A control program for dasheen mosaic virus in ornamental aroids is described; though performed in an idyllic situation in Florida normally unavailable for the Asian and Pacific taro growers, the discussion following the paper offers ideas on adaption of the techniques used in limited situations for the region.

Among fruit crops, grapes is said to be the most valuable on a world scale, and for the Asian and Pacific region it is the most rapidly expanding crop. Three phloem limited viruses, grapevine ajinashika virus (GAV), grapevine stunt virus (GSV) and grapevine leafroll virus (GLRV) are serious diseases of Japanese grapevines and believed widespread throughout vineyards of the globe. In grapes the ideal would be to identify the virus presence in the dormant stick. ELISA has been used successfully to identify viruses in the young plant and root stock phloem. Particle level observation has also been made, and could be used in a certification program.

The field of virology is highly technical as sophistocated techniques in the laboratory are essential for detection and identification of the viruses. This requirement often presents a significant obstacle to the less developed countries of the Asian and Pacific region in tackling virus disease problems or even recognizing them. A simplified practical method was given for using the direct fluorescence detection method. The section may be cut without microtone and used directly. Tissue must contain a vascular bundle. The detection technique is valuable for detecting the presence of MLO at an early stage of plant growth.

On citrus crops of the Asian and Pacific region, there is widespread incidence of citrus tristeza virus (CTV) affecting citrus sweet orange, buntans, Yuzu, tangelos, tangors and others. Symptoms are stem pitting and low fruit yield of small size. A large number of satsuma mandarins show no symptoms despite infection, and act as carriers of severe strains. Japan previously had a citrus industry based almost entirely (80-87%) on satsuma mandarin with trifoliate root stock. Consumer demand forced diversification on the industry, which led to the planting of many susceptible varieties of citrus. There is a great problem for self rooted trees used in breeding programs as these are particularly susceptible. Trees on the trifoliate root stock base though somewhat susceptible to stem pitting are not so susceptible to die back. In Korea the citrus industry of Cheju Island is exclusively founded on trifoliate root stock, and 97% of the crop is satsuma mandarin. In this situation tristeza would not likely appear as a problem according to the Japanese experience.

In Taiwan the disease 'likubin' has now been determined to be identical to the virus-like disease greening. Infected citrus are normally found to carry mixed infection with tristeza. The vector psylla, *Dixphorina citri,* of the greening disease, is in fact a very inefficient vector and the disease is bacterial rather than viral. It requires 21 days for the bacteria to develop in the gut of the vector; unfortunately however, control by insecticide spraying has to date proven impossible both in Japan and Taiwan. Through the means of top grafting Rusk citrange as an indicator, citrus tatterleaf has recently been shown to affect about 70% of Taiwan's citrus orchard trees. If the TLV virus could be eliminated from scion cultivars then the trifoliate rootstock can be used thus improving fruit quality. Methods of heat

therapy and modified shoot tip grafting are effective in cleaning the scion of TLV. Ponkan and Tankan in past years had been thought incompatible with trifoliate root stock, this has now been proven caused by TLV infection. If the TLV virus were elimated in mother-stock and certified budwood propagated, TLV not being a vector transmissible virus, orchard practices of dipping pruning shears in disinfectant may be helpful in control.

In citrus crops it was seen that there were the necessary elements of integrated programs in different places without their proper synthesis into complete programs; which was wasteful. In Japan there is great pressure for variety renewal to meet changing consumer demands which leads to the common practice of top-grafting. Without proper certification programs this provides a means for the spread of virus diseases. Scientists call for a certification program based on ELISA detection. Success is reported in eliminating virus and virus-like diseases in some experiment stations but without the controls reinfection occurs in the field from other citrus and other sources. Consequently, Japanese research has turned to development of cross protection with mild strains. A proposed program for the propagation of preinoculated budlines requires establishment of virus-free stock, raised in a screenhouse, with preimunized trees and also their first derivitives obtained through bud propagation also maintained in the screenhouse. The F_2 generation bud stock would then be used for propagation to the fields. Without the additional protection of raising the first derivitives in the screenhouse orchardists can expect aphid infection of the tree showing mosaic distribution of mild and severe strains.

Protection by mild strains is very host dependent; the most suitable variety of plant must be matched with a suitable mild strain of a virus to achieve optimum protection. Further mutation of severe strains to compete with the selected mild strain must be reckoned with by continuing further trials of alternative mild strains for each host plant variety. In such programs it is possible that scientists are looking at 10-20 years of work, therefore there is a search for a more expedient approach, enabling identification of the various virus strains through techniques simpler than the biological indexation methods in practice. The aim is to be able to identify viruses in early stages and check the severe strain infections.

In Thailand protection by tristeza mild strains has been reported and work is underway to propagate protected budwood. In the Philippines it was reported to the seminar that an integrated control program had been effective in one experiment station producing a 'greening' free orchard. Unfortunately the program had failed after five years of success when protection from insect reinfection was discontinued.

Papaya ringspot disease is a major regional problem. In Malaysia it was reported as widespread with orchards having 20-100% infection, with a similar picture in Thailand where attempts to select tolerant varieties have been unsuccessful to date. In the Philippines the spread of a new disease with ringspot type symptoms is reported. General identification so far points to ringspot virus of some kind. The experience of other countries of the region with this type of virus disease makes positive serological identification a priority for Philippine virologists in order to efficiently tackle this problem. Taiwan researchers provided a wealth of information on the ringspot problem specific to their situation. Taiwan has benefited enormously by cooperation in this field with American virologists, notably Dr.

D. Gonsalves. Less susceptible, tolerant varieties of papaya were initially selected though with lower yield than the preferred Tainung 2 variety. Mild strains of the virus utilizable for cross protection could not be found, but a mild strain was provided from Hawaii which is showing promising results in cross protection experimental trials using Tainung 2 variety.

The potential for control and eradication of the various viruses was a major concern to participants of the seminar. One approach is the breeding or selection of resistant varieties of plant. Another approach is eradication. In Khon Kaen Province northern Thailand attempts to eradicate papaya ringspot disease by burning all trees was unsuccessful. After six months of replanting the disease was observed in 80% of the new stock replanted. In Taiwan eradication of the papaya ringspot has not been attempted so far for practical reasons, including particularly the hostility of farmers to incineration of infected trees. Isolation is a third approach. Relocation of Taiwan's papaya industry on clean ground was attempted in 1980. The eastern lowlands, isolated from the traditional growing areas by a mountain chain were planted with healthy stock. Insufficient quarantine led to the inclusion of contaminated seedlings being planted in the area in the following year. Selection of a tolerant variety of plant had only fair success. Finally cross protection offers greater hopes. It is hoped that within three years the high producing variety, Tainung 2 now protected by mild strain preinoculation will again dominate Taiwan's growing areas.

Scientists attending the seminar stressed the need for an integrated virus control program, with eradication as the ultimate goal. In Hawaii on the island of Oahu, Dr. D. Gonsalves pointed out that control had been achieved on PRV through strict enforcement of the Department of Agriculture's eradication laws. With papaya ringspot it is necessary to have the possibility of isolating the crop to effect eradication. Therefore the program was protected by strict quarantine and even a backup of attenuated strain development in case the virus did ultimately invade the growing areas.

On an international level virus control programs need be coordinated, but the basis of any such program depends on respective countries practising strict quarantine controls. The controls must not only be effective in preventing the entry of unauthorized plant material having pathogens which are not present or present only at low incidence in the country; but, also important is to extend to plant breeders a system of authorized importation of safe indexed plant material. In some cases this merely precludes the importation of vegetated material and enables importation in seed form. In the case of the citrus industry it necessitates the establishment of a system for the importation of budwood that is pathogen free or which can be cleaned and indexed by authorities. The system now practiced in California as developed by Navarro *et al.* was reported to the seminar. As a system of excluding unauthorized plant material importation the extremely strict measures applied in Australia were recommended.

Peter W. MacGregor
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KEYNOTE SPEECH

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It is my great pleasure to have this opportunity to be with you this morning on the occasion of the opening of the Seminar on Virus Research of Horticultural Crops in the Tropics. Many seminars of a similar nature have been held in the temperate countries, but this is the first one to take place here in the Republic of China and to deal with crop disease problems important to the tropics and subtropics. I consider the holding of this seminar timely and useful because most countries in the ASPAC region are now faced with a rice surplus and are converting some rice acreage into cultivation of vegetables and fruit. The changing emphasis in cropping is accompanied by the emergence of new pest problems, including those due to viruses, which require research and extension work. I therefore would like to take this opportunity to commend FFTC and the Taiwan Agricultural Research Institute, co-sponsors of the seminar, for their insight and hard work in organizing this timely and purposeful scientific event.

I begin my talk with a quick sketch of Taiwan's geography that bears upon crop cultivation. The island of Taiwan has a total land area of about 36,000 square kilometers. The central mountain range runs from north to south, dividing the island geographically into two parts: the larger part lies on the west coast where about 97% of the population reside and intensive agriculture is practised; a smaller part on the east coast with only 3% of the population is cultivated less intensively and less diversified. With the tropic of Cancer passing through around the middle of the island, nominally Taiwan has a tropical climate south of Chiayi and a subtropical climate to the north. The yearly average temperature is 20°C for the plains, and the total precipitation ranges from 2,000 mm to 2,500 mm for the plains, and is higher for the mountains. It is therefore not surprising that the major horticultural crops of Taiwan include tropical, subtropical and temperate crops. To list just a few, they include bananas, papayas, mangos, wax apples, sugar apples, litchis, longans, citrus, loquats, grapes, peaches, pears and apples. In recent years, the application of new horticultural technology has made it possible for growers to derive two or three harvests from some of these crops encouraging increased planting; notable are grapes and wax apples, with a three fold and a ten fold increase in planted area respectively, during the last decade. Taiwan's agriculture may not be the specific topic of this seminar, but I wish to point out the extremely wide variety of economic crops being grown here and hence the complexity of pest problems that our researchers and farmers encounter.

Plant diseases caused by viruses are indeed a worldwide problem in crop production. Virus infection may lead to visually recognizable, severe symptoms or it may not be expressed externally. However, in either case infected plants may suffer losses in yield and quality to varying degrees. Chemical controls have to date not proved particularly useful. Virus diseases are better controlled by prevention, by use of resistant varieties, virus-free seed and stock and other preventive measures. A sound control program also calls for close cooperation on district and regional levels in the selection of crops, their varieties to be grown, the timing of planting, the suppression of vector activities, and the enforcement of field sanitary measures for the removal of infection sources. The involvement of government agencies is essential. I shall illustrate this point by citing two major virus control programs that the government of the Republic of China has implemented. First a field control program on rice virus and virus-like diseases during the 1970's; transitory yellowing due to virus and yellow dwarf due to an MLO caused great losses to the second rice crop

in the 1960's and early 1970's. Both are spread by the rice green leafhopper. A government-subsidized program aimed at the control of vector insects was implemented in 1971 and continued through to 1979, with 16,000-70,000 ha of rice fields aerially sprayed annually. By the late 1970's, these leafhopper-borne diseases were so insignificant that virtually no crop losses resulted from either. Varietal resistance did not appear to be a contributing factor in the reduced disease incidence because no useful resistance sources were found or utilized. This program is now discontinued. Second a program for citrus virus and virus-like diseases with the goal of supplying healthy seedlings of commercial varieties for use by growers in Taiwan. Two parallel approaches have been taken: the establishment of nucellar lines, begun in 1976 and use of the so-called micrografting technique begun in 1981. Some of the better nucellar lines have already matured and are ready for multiplication. From the micrografting program, about 8,000-10,000 young healthy seedlings of four major citrus varieties will be released in 1986. Thus growers will have access to planting materials certified free of virus or virus-like organisms.

Plant quarantine is another important aspect of government involvement in virus disease control strategies. In the past we have witnessed the invasion with some frequency of plant viruses devastating our crops. The papaya ringspot virus is the most noticeable case. The virus was not known to Taiwan's papaya growers until 1975 and then spread to nearly all papaya orchards in the main production areas in less than three years. It has been a focus for research activities and control programs ever since. However, our concern about plant quarantine is not only because of past experiences but also because of the increasing risk of invasion and dissemination of plant viruses associated with the growing quantities of plant materials that are imported and exported. According to recent statistics, Taiwan is exporting 700,000 kg of vegetable seeds to countries in Europe, East and Southeast Asia and the Americas annually, while it is importing 600,000 kg annually from these areas. A strict enforcement of plant quarantine procedures will be of great benefit not only to the plant industry of Taiwan but to our trading partners as well. It is in this context that the Council of Agriculture has taken steps towards further strengthening plant quarantine in both regulatory and operational aspects.

Close cooperation between plant virus researchers internationally has proved mutually beneficial. Sources of virus resistance in plants can be exchanged for use in breeding programs. Virus-free stocks shipped as test-tube plants can be propagated in any country, a technique of immeasurable value. Monoclonal and polyclonal antibodies with specificity to a virus or virus strain produced in one laboratory will find application elsewhere. Fragments of viral genome or their complementary sets are among the most powerful probes for the purpose of virus identification and disease diagnosis. Their shipment is simple. Of late, mild strains of plant viruses have been shown to be capable of protecting host plants under field conditions against superinfection with severe strains of the same viruses. Exchanges of mild strains are very much to be urged between laboratories, institutions or countries concerned with common virus problems. Cooperation of this kind exists between Cornell University and agricultural institutions in Taiwan. Two mild strains that were artificially induced from a field, severe strain of the papaya ringspot virus at Cornell University have been introduced into Taiwan for field control trials. Although it is still too early to say that the mild strains may be an answer to the papaya ringspot problem, two years of field experiments demonstrate a level of disease control that surpasses any other control measures presently available. We released a total of 200,000 mild strain-protected papaya seedlings for fall planting in 1984. For planting in this fall, 710,000 such seedlings are to be released. I urge more international cooperation of this kind in the future.

We must give due attention to research of a basic nature based on plant virus materials. Plant viruses provide opportunities for researchers in their quest into basic biology. Viruses are of great value in the study of gene actions, and have potential as a vehicle for desirable genes to be incorporated into plants of economic importance. Those with interest in genetic engineering may find many other uses for plant viruses. As far as plant virus research is concerned, we in the government strive to maintain a healthy balance between what is problem-solving and what is basic pure research.

SECTION I

NATIONAL PLANT VIRUS SITUATION KOREA, MALAYSIA AND THAILAND

VIRUS DISEASES OF HORTICULTURAL CROPS IN MALAYSIA

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SUMMARY

Many horticultural crops in the Solanaceae, Cucurbitaceae and Fabaceae are seriously affected by virus diseases. Some viruses are widespread throughout the Malaysia. However, diagnosis or identification of most of the viruses involved are incomplete and long overdue. More information on their transmission characteristics and ecological relationships have to be obtained. Control measures are greatly lacking.

摘　要

茄科、葫蘆科與豆科之園藝作物均遭受到病毒之嚴重感染，許多病毒已普遍的在國內各地發現。主要病毒的診斷或鑑別雖爲重要之工作，但仍未能完成。因此必需要有更多有關病毒傳播特性與生態關係之資訊。目前有關防治之方法更是缺乏。

摘　要

マレーシヤにおいては、ナス科、ウリ科、マメ科の多くの園藝作物のウイルス病の被害は甚大であり、そのあるものは全國に廣く分布している。しかし、大部分のウイルス病については診斷や同定は不完全で長く立遲れている。これらのウイルス病の傳搬特性や生態學的關係についてもつと知識をもつ必要がある。防除法は大きく欠落している。

VIRUS DISEASES OF HORTICULTURAL CROPS IN MALAYSIA

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INTRODUCTION

Horticultural plants in Malaysia comprise more than 50 vegetable, 100 fruit and numerous ornamental (including flower) species[8,9,12,18]. However, only a small number are planted on an intensive or commercial scale. Most occur in mixed cropping patterns of less than a few hectares[3,4].

Among the virus diseases listed in the country, 20 or so are associated with horticultural plants[15,22]. Lately, a few more have been added[1, 13,24,25] and this may increase further if a thorough survey is conducted [6,10]. With the import of seeds and other planting materials increasing each year[21] the problem may further be aggravated by the inadvertent entry of other viruses through such sources.

Much of the information available on the local virus diseases so far are incomplete. In many cases the etiology and mode of transmission (including vectors involved) have not been sufficiently studied. There has been very little work done on epidemiology and control. Within the last few years the problem appears to have escalated especially in solanaceous, leguminous and cucurbitaceous crops. The diseases have become severe and widespread. Heavy losses have occurred in crops of chillies[20] and cucurbit fruits. A more systematic approach to the whole problem is justified.

CROPS STUDIED FOR VIRUS DISEASES

Solanaceae

Chillies (and peppers), *Capsicum* spp., often suffer from very destructive diseases. Field symptoms are at times complicated by mites, thrips or other insect infestations. Various forms of mosaic-mottle, leaf distortion or malformation and necrosis are often seen. Symptoms on the fruit range from chlorotic to necrotic patterns, skin roughness and distortion or deformation. Flowers may also be malformed or aborted. Therefore, both yield and fruit quality can be affected.

The incidence of virus infections in chillies seems to be widely distributed throughout the country. The early virus reports include cucumber mosaic, tobacco mosaic and pepper (chilli) veinal mottle viruses[11,15]. Current observations also suggest the presence of tomato spotted wilt virus[13], an unidentified potexvirus[25] and potato virus Y[6]. Isolates with symptoms and certain test plant reactions similar to alfalfa mosaic and potato virus X are presently also undergoing further tests. Mixed infections of different viruses appear to be of common occurrence.

Transmission studies so far confirm aphid vectors of pepper veinal mottle and cucumber mosaic viruses[15]. In the case of other viruses affecting chilli, despite much speculation of seed transmissability and the involvement of other vectors, especially arthropods like thrips, whiteflies and mites, there appear to have been no attempts to confirm these so far. For pepper veinal mottle virus, the importance of winged aphids and differences in transmission efficiencies among seven aphid species have been demonstrated[16]. Reflective mulches have been shown to be effective against the spread of the disease but their disadvantages still outweigh their usefulness[14]. Lately, more attempts to search for resistant

strains to the virus have been reported[11].

In tomato, *Lycopersicon esculentum* Mill., various syndromes similar to those caused by virus infections have been observed in the field. These include various degrees of stunting, top bunching or bushiness leaf curling or cupping, twisting, malformation and necrosis. Symptoms may also appear on fruits and fruit size may be affected. The disease caused by tobacco mosaic virus was recorded some time back[15]. Recently, however, potato Y, cucumber mosaic, tomato spotted wilt and tomato yellow leaf curl diseases have been recognised or suspected. A disease similar to that of potato spindle tuber or tomato bunchy top in symptomatology and host range reactions has also been detected. It was found to occur in about 5% of tomato plants raised from imported seeds. Interestingly, although pepper veinal mottle virus is widespread in the country, it has neither been isolated nor infective to tomato, contrary to widespread reports of this type of virus elsewhere[7].

In eggplant, *Solanum melongena* L., tobacco mosaic virus infection was reported[15]. Recently, another disease showing mosaic and necrotic ringspots was observed. Cucumber mosaic virus is believed to be associated with the syndrome (*Fujisawa, personal Comm.*). Current observations have further showed that plants inoculated with an isolate of potato virus Y from chilli also produce systemic reactions. These appear as mild mottling and faint chlorotic ring and line patterns. Certain African strains of pepper veinal mottle virus are known to infect eggplants as well[7], but the local isolate apparently does not[11,17] which therefore may be quite similar to the isolate reported in India[19].

In terms of ecological relationships, it is not unusual to find virus infected or susceptible weed and alternative crop hosts in the field but their role in epidemiology of the diseases have not been elucidated. Virus infections (like cucumber moscaic and tobacco mosaic) have been detected to occur naturally in the weed, *Physalis minima* L. Other weeds suspected to harbor viruses include *Solanum nigrum* L., *Ageratum conyzoides* L., *Commelina nudiflora* L. and *Amaranthus* spp.

Cucurbitaceae

Cucumber mosaic virus is probably common throughout the country but apparently seldom a problem in cucumber, *Cucumis sativus* L. Recently, pumpkin, *Cucurbita maxima* Dcne., with severe mosaic symptoms has been observed. It is believed to be associated with zucchini yellow mosaic virus (Fujisawa, personal comm.). Severe mosaic mottle and stunting syndromes have also been seen in watermelon, *Citrullus lanatus* (Thunb.) Mansf., and squash, *Cucurbita pepo* DC. Limited tests seem to indicate some similarities to watermelon mosaic virus infections. An incidence and spread of a chlorotic-necrotic spot disease was recently found in a trial plot of introduced muskmelon, *Cucumis melo* L., varieties.

Fabaceae

A review of virus diseases of legumes has been presented elsewhere[2]. Yardlong bean, *Vigna sesquipedalis* (L.) Verdc., has been seen to suffer from a number of virus infections. Symptoms observed include various forms of chlorotic or yellow mosaic mottle and distortion. An aphid-transmitted virus causing the disease 'longbean mosaic' was reported to be seed-borne up to 50%[15]. Another virus isolated recently is the blackeye cowpea mosaic[1,24] which is vectored by aphids and also seed-transmissable at a low rate. Current observations seem to suggest that certain other unidentified viruses may also be seed-borne.

French bean, *Phaseolus vulgaris* L., appear to be fairly free from destructive virus infections. However, sporadic incidences of a mild mosaic and a crinkling stunt diseases have been seen. Etiology of these diseases has not been studied. A severe rugose mosaic isolate from bean which when sap-inoculated onto chilli produced mosaic mottle symptoms has been studied recently. A chilli isolate from an adjacent plot was also infectious to bean producing similar symptoms as the bean isolate. Studies are continuing to determine whether the diseases are of the same etiology.

Leafy vegetables

Virus diseases of leafy vegetables have been mentioned in previous publications[15,22]. Additionally, mosaic mottle symptoms have been seen in Chinese spinach (*Amaranthus* spp.) and sweet shoot (*Saurapus albicans* Blume) but so far their distribution has not been determined and etiological studies have yet to be made.

Citrus and other fruit crops

Previous publications[15,23] have listed most of the virus diseases found in the fruit crops. Presently, they appear to be of little concern to the growers.

Ornamental and flower crops

Very few virus diseases have been found in ornamentals[15,22] and they do not seem to adversely affect the plants.

CONCLUSION

A systematic approach and a more intensive effort are required to solve virus disease problems in Malaysia. Urgent attention should be given to virus identification or diagnosis, transmission characteristics, ecological relationships and control measures.

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VIRUS DISEASES OF HORTICULTURAL CROPS IN THAILAND

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SUMMARY

There are at least five important virus diseases of horticultural crops in Thailand. Tomato yellow leaf curl, tomato fern leaf, tristeza of lime and ringspot of papaya are caused respectively by tomato yellow leaf curl virus, cucumber mosaic virus, citrus tristeza virus and papaya ringspot virus. Mosaic and little leaf of pepper is caused by mixed infections of cucumber mosaic virus, tobacco mosaic virus and pepper mottle virus. Although, eradication is shown to be a possible control method for papaya ringspot disease, it is probable that such results will only be obtained with cooperation of understanding growers. So far no resistant plant varieties exist among those crops, researchers are looking for mild protective strains of those viruses. Mild strains of citrus tristeza virus in lime have been reported.

摘　要

在泰國至少有五種以上之病毒爲害園藝作物。番茄黃色捲葉病，細葉病，南美立枯病和木瓜輪點病卽分別由番茄黃色捲葉病毒、胡瓜嵌紋病毒、柑橘黃葉病毒與木瓜輪點病毒所造成。番椒的嵌紋與細葉病徵是由胡瓜嵌紋病毒、菸草嵌紋病毒與番椒斑點病毒混合感染造成。拔除病株雖然爲木瓜輪點病之可能防治方法，但必需要取得農家的合作與諒解。至目前爲止。尚未有品種具有抗病性，許多研究人員已開始尋求弱病毒品系。而且已有有關菜母之南美立枯病弱病毒品系之報告。

摘　要

タイ國の園藝作物には少なくとも 5種類の重要なウイルス病がある。卽ち、tomato yellow leaf curl virus によるtomato yellow leaf curl病、cucumber mosaic virus によるtomato fern leaf病、citrus tristeza virus によるlimeの tristeza病、papaya ringspot virus によるpapaya ring spot 病である。トウガラシのモザイクや little leaf 症狀はcucumber mosaic virus, tobacco mosaic virus およびpepper mottle virus の重複感染によつておこる。papaya ring spot 病を防除する 1方法に病株の拔取があるが、これは栽培家の理解と協力があつてはじめてなしとげられる。現在の知識ではこれらの作物の中に抵抗性の品種はなく、研究者はこれらのvirus の mild strain をさがしている。limeの tristeza virus についてはmild strain の發見が報告されている。

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INTRODUCTION

Six viruses identified to cause considerable yield losses in important horticultural crops in Thailand are tobacco mosaic virus[8], cucumber mosaic virus[11,13,17,18], pepper mottle virus[15], tomato yellow leaf curl virus[1,4,12,16], citrus tristeza virus[3] and papaya ringspot virus[2,5,6,9,10]. Current researches on these viruses are reviewed in this paper.

Tomato Yellow Leaf Curl disease

Caused by a geminivirus[12] it causes great yield loss in tomato crops growing in Bangkok, Nakhon Pathom, Samut Sakhon, Nakhon Ratchasima, Khon Kaen, Chiengmai, Lampang and Prachuab Khiri Khan Province[4,12,16]. Between 10 to 100% of this disease incidence was reported in these tomato growing areas[4]. Tomato yellow leaf curl virus causes curling of young leaves and they remain small. Lower leaves are curled, twisted, puckered and become yellow. The plants become stunted and bushy. Flower buds abort[4]. The virus is transmitted by the white fly, *Bemisia tabaci* in the persistent manner[1], but not by inoculation with sap. In transmission using the vector, the experimental host range is limited to three species in solanaceae, i.e. *Lycopersicon esculentum, Nicotiana glutinosa* and *Datura stramonium*[16]. No resistant varieties of tomato were found among 14 varieties tested[14,16].

Fern Leaf of Tomato

Fern leaf disease of tomato is caused by a virus serologically related to cucumber mosaic virus Y strain[13]. Found in all tomato planting areas in Thailand, it has become epidemic since 1981 in Chiengmai, Lampang and Nong Khai[13]. The virus has a wide host range and is transmitted by the aphid, *Myzus persicae* in the non-persistent manner and by inoculation of sap but not by seed[11]. It causes systemic mosaic, narrowed leaf laminae (fern leaf) and stunting of plants. Yield of infected plants is decreased and fruits are small[13]. Commercial tomato varieties growing in Thailand, i.e. Porter, L22, VF 145, VF 134-1-2 are all susceptible[11,13].

Mosaic and Little Leaf of Pepper

Commercial varieties of pepper (*Capsicum* spp.) growing in Thailand are infected with at least three identified viruses. Cucumber mosaic virus frequently occurs in pepper in mixed infections with tobacco mosaic virus and pepper mottle virus[8,15,17,18]. These viruses occur in peppers throughout pepper growing areas usually with high rates of disease incidence, causing systemic mottling followed by curling and distortion of leaves. Young leaves remain small. Fruits are distorted. Infected plants survive but yield is low.

Tristeza of Lime

In Thailand, tristeza is the most destructive virus disease of lime (*Citrus aurantifolia*) which is grown as airlayers. The virus causes vein clearing pattern (enation) and cupping of leaves. Diseased trees show dieback of twigs, dulling and dwarfing of foliage, and reduction in size and number of fruits[3]. According to Knorr *et al.*[3], because of the

widespread distribution and high population levels of the efficient vector, *Toxoptera citricida* in Thailand, it is likely that all citrus trees in the country contain the virus of tristeza. It is believed that passive immunization may occur naturally. For control they suggest that growers select only the best trees for making airlayers.

Papaya Ringspot

Papaya ringspot, caused by papaya ringspot virus (PRV)[10] is the most destructive disease of papaya (*Carica papaya*) which is grown mostly throughout the Northeast of Thailand[2,5,9]. A survey conducted by Prasartsee *et al.*[6] showed that that papaya in 15 of 17 provinces in the Northeast of Thailand were infected with PRV and the disease incidence varied among fields 20-100 percent. In papaya, the only natural host, the virus causes mottling and distortion of leaves, streaks on stems and petioles, rings and spots on fruits and stunting of plants. Yield of infected plants is much decreased[2,6]. PRV is transmitted by three species of aphids, *Aphis gossypii, A. craccivora* and *Hysteroneura satariae*, in a non-persistent manner but not through seeds[2,5,9]. All attempts to select PRV resistant varieties were unsuccessful. All of 22 varieties of papaya tested were susceptible[6]. An eradication program was conducted at four severe PRV infected villages in Khon Kaen Province[5,6]. All papya plants growing in these villages were incinerated. After four to five months healthy seedlings were introduced and replanted. The results showed that six months after replanting only one of those four villages was absolutely free from PRV infection.

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DISCUSSION

Q. (M. Koizumi)
To what extent do you think that greening disease is responsible for the decline in limes?

A. (S. Attathom)
The situation of greening in the limes, is not as serious as in sweet orange, we have put some greening agent into the limes and they appear to be very resistant. So the decline in lime is not expected due to greening disease.

Q. (M. Koizumi)
The symptoms of Mexican lime seedlings inoculated with tristeza virus in Thailand do not seem severe. This suggests that there are pathogens responsible for the decline of acid lime other than tristeza which should be considered. Greening disease is wide spread throughout the region in citrus and combined injury due to tristeza and greening is possible. To what extent do you think greening may be responsible for the decline seen in acid limes in Thailand?

Comment: (C.N. Roistacher)
The symptoms shown of tristeza did not appear too severe, I would have expected vein corking and stem pitting. Do you see the severe vein corking symptoms in Thailand?

A. (S. Attathom)
We do not see much stem pitting in Thailand, except in some pomello and mandarin trees that show a minor stem pitting symptom. The CTV symptoms on limes seen in the slides shown were only of young seedlings. We do see severe symptoms in the orchards and decline of trees.

Comment: (M. Koizumi)
I have had some experience with greening disease seen in Bangladesh, the acid lime can be resistant against greening, but that plant usually carries the disease in the tissues. There the symptoms of greening appeared severe during the hot season and it was responsible for the decline of trees. In Thailand since symptoms of tristeza are not severe, there is need to concentrate research work on the combination of the two and their relationship.

A. (S. Attathom)
What Dr. Koizumi is suggesting is that lime is perhaps a reservoir of greening in Thailand. We will keep an eye on that.

Q. (F.W. Zettler)
I noted that the distribution of cucumber mosaic virus in tomato was in the north near the boarders of Burma and Laos. Is that also true of pepper, and is there any correlation between the distribution of CMV and the cooler climates?

A. (A. Chandrasrikul)
It is in fact spread very widely; however, we have not surveyed this thoroughly as we lack the facility of a quick test.

Q. (O.S. Opina)
In the Philippines we have a problem of papaya ringspot virus. What is the status of research on papaya ringspot disease in Thailand?

A. (A. Chandrasrikul)
We recommend that farmers noticing the disease, incinerate all infected plants and reintroduce clean plants after three or four months. At this stage we have no resistant variety.

Q. (O.S. Opina)
Papaya ringspot is not seed transmitted to papaya; however, you have mentioned that it can infect cucurbits (water melon). In these is it seed transmitted?

A. (N. Deema)
No, it is not.

VIRAL DISEASES OF HORTICULTURAL SUBTROPICAL AND TROPICAL CROPS IN KOREA

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ABSTRACT

Cultivation of tropical and subtropical crops in Korea is mainly limited to the island of Cheju. Virus diseases of citrus are a minor problem, those identified include satsuma dwarf virus, exocortis and tristeza, however, a program is planned to keep these in check. Bananas and pineapples are grown under greenhouse conditions, though quality is poor due to climatic restrictions. Kiwi fruit, a newly introduced crop shows promise on higher slopes.

摘　要

在韓國熱帶與亞熱帶作物之栽培僅限於濟州島。柑桔毒素病並不嚴重，已知的有Satsuma矮化病毒，Exocortis，與柑桔南美立枯病，並已成立檢定之計畫。香蕉與鳳梨均種植於溫室內，受到氣候之限制，品質不佳。獼猴桃爲一新興作物，在較高之山坡地上具有潛力。

摘　要

韓國における熱帯、亞熱帯性植物の栽培は主として濟州島に限られている。柑橘のウイルス病として温州萎縮、エキソコーテイス、トリステザがあり、あまり重要ではないがこれらをチェツクする計畫がたてられた。バナナ、パイナツプルは温室栽培され、氣候條件が悪い爲品質はよくない。キゥーイフルーツが新に導入され有望である。

VIRAL DISEASES OF HORTICULTURAL SUBTROPICAL AND TROPICAL CROPS IN KOREA

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INTRODUCTION

The cultivation of tropical crops in Korea is limited to the Island of Cheju and the southern coast of Korea.

Cheju Island, the largest and southernmost island in Korea, is located about 100 kilometers south from the mainland, lat. 33°N. Mt. Halla, 1950 meters high dominates the center of the island. The mean annual temperature is around 15°C and winter minimum temperature 2.3°C. The annual rainfall ranges 1,440 to 1,718 mm, the number of days with wind velocity of more than 8 m/sec damaging to some tropical crops is 90 to 150. Infertile valcanic ash soil with a high coefficient of phosphate absorption predominates Cheju's soils.

Despite these unfavorable environmental conditions, some tropical and subtropical crops are successfully grown on the open field or in heated plastic houses during winter. These crops grown on an economic scale in Cheju Island include citrus, pineapple, kiwi fruit, and banana; while feijoa, pepino, papaya and guava are experimentally grown.

SUBTROPICAL & TROPICAL CROP CULTIVATION IN CHEJU

Tropical and subtropical crops occupy a large percentage of Cheju Island's cultivated acreage (below 300 m). The acreage of tropical and subtropical crops amounts to 35.5% of upland croping area of which citrus occupies 99% of the total (Table 1).

Table 1 Use of arable land below 300 meters above sea level on Cheju

Item	Total	Upland crops			Paddy fields	Pasture	Others
		Tropical & subtropical	Others	Total			
Area (ha)	122,667	17,180	31,272	48,452	1,059	39,298	33,858
Ratio (%)	100	14.0	25.5		0.9	32.0	27.6
Upland Crop Ratio (%)		(35.5)	(64.5)	(100)	—	—	—

Cheju Province Statistics

Citrus

Historically, Cheju Island has supplied citrus fruits to the mainland even before 1053 A.D. In the late 1960's, many more farmers joined the commercial citrus industry of this island, and since 1970 there has been a great increase in citrus production (Table 2).

Satsuma mandarin (*Citrus unshiu* Marc.) introduced about 70 years ago now represents 97% of the citrus industry in Cheju. Trifoliate rootstock is used exclusively.

Citrus growing is the leading industry in Cheju and a major Korean fruit crop. (Table 3)

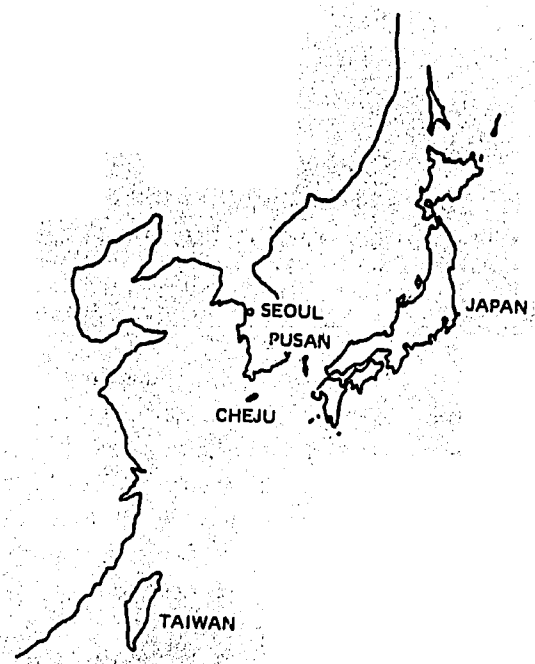

Fig. 1 Location of Cheju Island

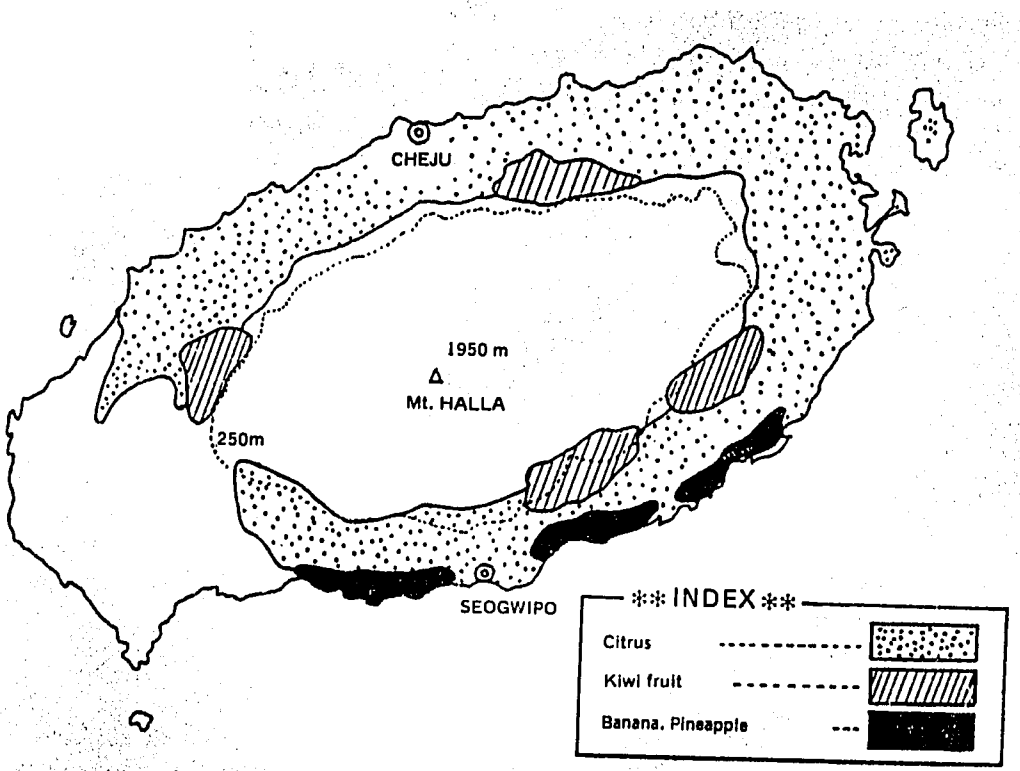

Fig. 2 Distribution of subtropical and tropical crops in Cheju Island

Table 2 Increase in citrus production

Year		1970	1975	1980	1984
Production	(1,000 ton)	4.9	81.1	187.5	261.0
Acreage	(1,000 ha)	5.0	10.9	14.1	17.0
Productivity	(ton/ha)	0.9	7.4	13.3	15.4

Cheju Province Statistics

Table 3 Fruit industry statistics 1983

	Acreage	Production	Ratio
Apple	41,297 ha	586,023 M/T	41.9 %
Citrus	14,838	330,623	23.6
Pear	9,802	106,304	7.6
Peach	10,732	99,199	7.1
Grapes	14,165	131,111	9.4
Others	14,635	146,448	10.4
Total	105,469	1,399,708	100.0

Ministry of Agriculture and Fishery

The most troublesome diseases and insects are as follows:

Insects: Major – Citrus red mite (*Panonychus citri* McGregor)
– Red wax scale (*Ceroplastes rubens* Maskell)
Minor – Citrus leaf-miner (*Phyllocnistis citrella* Stainston)
– Citrus rust mite (*Aculops pelekassi* Keifer)
– Citrus whitefly (*Dialeurodes citri* Ashmead)

Diseases: Melanose (*Diapirthe Citri* Wolf)
Scab (*Elsinoe fawcetti* Bitan Court et Jonkins)
Canker (*Xanthomonas citri*)
Virus diseases

All are effectively controlled by agricultural chemicals except the citrus red mite which shows strong resistance to the available chemicals.

Citrus viruses have been studied in Cheju Island since 1980. The characteristic symptoms caused by citrus viruses show that about 4.5% of the citrus trees have satsuma dwarf virus (SDV) disease; symptoms of boat-shape, spoon-shape and rosette-type leaves were observed. Tests with indicator plants such as sesame proved that those symptoms were caused by satsuma dwarf virus.

Exocortis viroid and tristeza viruses were also observed. When Hassaku trees infected with tristeza virus were treated for 50 days at 40°C day and 30°C night temperatures the shoot-tip of the treated tree was virus free to fluorescence microscope inspection.

Viruses are not yet recognized to cause serious problems in the Cheju citrus industry; however, a program is planned to overcome the possible diffusion of virus diseases, including the establishment of virus testing and the maintenance of virus-free strains.

Banana

Banana was first introduced into Cheju Island in 1977 when a farmer imported dwarf cavendish from Taiwan to cultivate in a 500 m^2 plastic house. Its cultivation has been increased remarkably since (Table 4).

The farmers must grow bananas in plastic houses heated during the cold winter.

The production cost is offset by cash sales to tourists, and acreage of banana cultivation is expanding.

The typhoons in July and August, and high winds during winter sometimes demolish the plastic houses, destroying the farmers whole crop. Farmers plant windbreaks and are improving the plastic houses.

The short history of banana cultivation means the crop is relatively free of any insects and diseases. The first investigation of the nematode density performed this year, however, has warned us to forestall the prospective danger of the banana nematode (*Radopholus similis*) (Table 5).

Banana virus diseases are suspected. Some large-scale farmers are raising banana seedlings by means of tissue culture, replacing their old plants and supplying other farmers.

Pineapple (Ananas comosus Merr*)*

Pineapples grown since 1966, are now planted in about 90 ha of plastic houses (Table 6). Leading cultivars are special Amarelo and Sarawak (Table 7).

Table 4 Increase in banana production, Cheju

		1981	1982	1983	1984	1985
Production	(ton)	10.2	36.0	58.0	319.0	—
Acreage	(ha)	0.5	2.0	3.8	13.0	30.0
Productivity	(ton/ha)	20.4	18.0	15.3	24.5	—

Cheju Province Statistics

Table 5 Density of banana root nematode in 100 g of banana orchard soil (1985)

Banana orchards				Mean
A	B	C	D	
161.4	165.4	245.6	21.0	148.4

Table 6 Pineapple plantations

		1981	1982	1983	1984
Production	(ton)	2,015	2,620	1,653	3,172
Acreage	(ha)	92	99	87	90
Productivity	(ton/ha)	21.9	26.5	19.0	35.2

Cheju Province Statistics

Table 7 Acreage of pineapple cultivars, Cheju

		Sarawak	Special Amarelo	Tae-Nong 5	Perote	Total
Acreage	(ha)	45.6	35.8	5.5	0.1	87.0
Ratio	(%)	52.4	41.2	6.3	0.1	100.0

Cheju Experiment Station

Fruit quality is not good, however, fruits being sourer with high acidity, low sugar and light in weight (Table 8).

Pineapples are simply protected by two or three layer polyethylene films without heating in the winter, which is not sufficient to keep air temperature above 16°C, the minimum growth temperature for pineapple. Reduced solar radiation due to thicker polyethylene film is a contributing factor to the low quality of pineapple grown in Cheju Island.

Tourists purchase pineapples as souvenirs rather than for table. To improve fruit quality an economic heating system using solar energy effectively, needs to be developed.

Kiwi Fruit (Actinidia chinensis Plench*)*

Kiwi fruit is one of the most promising newly introduced fruits. Grown since the end of 1970, it was only in 1980 that cultivation began on an economic scale.

Kiwi fruit production is increasing (Table 9). It can be grown on fields 250 to 300 meters above sea level where citrus can not survive. At this altitude wind damage is a problem even to the kiwi fruit without wind protection the plant will not yield fruits economically (Table 10).

Table 8 Pineapples cultivar fruit quality 1979-1983

		Sarawak	Special Amarelo	Tae-Nong 5
Sugar cont.	(%)	13.1	15.1	11.0
Acid cont.	(%)	1.68	1.64	1.32
Fruit wt.	(kg)	1.34	1.34	1.43

Cheju Experiment Station

Table 9 Yearly production of kiwi fruit

		1981	1982	1983	1984
Production	(ton)	—	7.8	25.0	62.0
Acreage	(ha)	3.9	11.0	28.0	71.5
Productivity	(ton/ha)	—	0.7	0.9	0.9

Cheju Province Statistics

Table 10 Kiwi fruit typhoon damage

	Typhoon Kity (Aug. 9-10, 1985)	Typhoon Lee (Aug. 13-14, 1985)
Wind velocity	17.3 m/sec	16.7 m/sec
Shoots & Twig damage	2.8%	unrecorded
Leaf damage	41.6%	44.2%

Cheju Experiment Station

DISCUSSION

Q. (M. Koizumi)
You have noted that there is virus in the citrus crops on Cheju island; what method do you use to prove this?

A. We index through sesame.

Q. (M. Koizumi)
Which is the type more noticeably infected with SDV, the early or ordinary variety of satsuma?

A. The early type.

SECTION II

VIRUSES OF VEGETABLE CROPS

VIRAL DISEASES OF CUCURBITS AND SOURCES OF RESISTANCE

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ABSTRACT

A number of viruses are known to infect cucurbits, here attention is given to those of great economic importance, and sources of resistance that are presently available cre listed. For convenience, the viruses are grouped according to their vectors, and sources of resistance will be given for cucumber, melon, squash, watermelon, bottlegourd, loofah, and waxgourd.

摘　要

本文列出數種已知會感染瓜類病毒之重要性及其抗病材料。爲了方便起見，病毒依其媒介體而歸類，並說明胡瓜、甜瓜、南瓜、西瓜、扁蒲、絲瓜與冬瓜等作物之抗病材料。

摘　要

ウリ科植物を侵す多くのウイルスがあるがこ、では經濟的に重要なものに注目し、現在抵抗性遺傳子源が利用できるものを表にのせた。これらの諸ウイルスを、便宜上、媒介者によつて分類し、キウリ、メロン、セイヨウカボチヤ、スイカ、ヒヨウタン、ヘチマ、トウガンの抵抗性遺傳子源を上げてある。

VIRAL DISEASES OF CUCURBITS AND SOURCES OF RESISTANCE

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INTRODUCTION

Most of the cultivated cucurbit species originated in tropical and sub-tropical regions of Africa, the Americas, and Asia. They constituted very important crops at the beginning of agriculture and continue to contribute substantially to the welfare of many regions of the world[72]. Like many other cultivated plants, cucurbits are constantly subjected to adversities, pests, and diseases. Some of the most damaging are viral diseases, which often reduce the quantity and quality of their yield. However, the incidence and severity of these diseases may vary through the years, depending upon the complex relationships among viruses, hosts, vectors, and environmental factors.

For years, eradication of overwintering hosts of the viruses and chemical control of the vectors have been the principal means of control. These methods continue to play an important role, and will supplement other biological and cultural practices, but it must be recognized that they are only partially effective and need to be repeated yearly, adding to production costs. Consequently, given the difficulty of controlling viral diseases by adopting preventive or curative measures, it is logical to expect that heritable resistance is the ultimate solution.

Sources of resistance to viruses can be found in:

1) existing cultivars,
2) primitive cultivars or land races,
3) closely related species, or
4) other genera of the same botanical family.

The first two sources can be quickly exploited and thus are more appealing, whereas those deriving from other species are often difficult to transfer because of genetic incompatibility or close linkage with undesirable characters. However, increasingly we are forced to rely upon wild cucurbit species as sources of resistance to viruses.

In contrast to sources of resistance to fungal and bacterial pathogens, genes conferring resistance to viral infections have been proven rather stable. However, in some cases, resistance is viral strain-specific. Thus, a resistance gene may confer resistance only to a specific strain (pathotype) of a given virus, necessitating an accumulation of genes for the control of all known pathotypes. When possible, the search for resistant germplasm should be made before these diseases reach catastrophic proportions, since the incorporation of resistance into horticulturally desirable cultivars requires several years of intensive breeding. Furthermore, unless the nature of resistance and its limitations are well understood, considerable wasted time and effort can be expended by cucurbit breeders.

VIRUSES TRANSMITTED BY APHIDS

Cucumber Mosaic Virus (CMV)

A cucumovirus with 3 functional pieces of single-stranded RNA included in 3 classes of isometric particles about 28nm in diameter, it is transmitted in a stylet-borne manner (non persistent) by more than 60 aphid species, which acquire the virus within 5-10 seconds and retain it for about 2 hours. CMV is easily transmitted mechanically and it has been reported to be seed-borne in 19 plant species, however, none in the Cucurbitaceae. This virus is of worldwide distribution, thus it occurs in the tropics as well as in temperate and cool regions, where it is able to infect more than 800 plant species. A number of

strains, pathotypes, and serotypes have been described. In cucurbits, it causes a prominent mosaic, leaf reduction, cupping, rugosity, and plant stunting. Fruits are also affected by distortion and discoloration[1,11].

Resistance:

Cucumber *(Cucumis sativus)*

The first evidence of resistance to CMV was reported in 1928, in the oriental cucumbers, Chinese Long and Tokyo Long Green[38]. Research on the inheritance indicated that resistance was conditioned either by 3 dominant[56] or 3 partially dominant[23] genes and possibly some modifiers. Conversely, another study concluded that resistance was due to a single dominant factor[67], to which the symbol *Cmv* was later given[49]. These studies have pointed out that in breeding for resistance, it is possible to select plants with varying degrees of resistance depending upon the number of genes involved. In general, CMV resistance in cucumber is better expressed under field than greenhouse conditions. Currently, most of the American cultivars are resistant to this virus.

Melon *(Cucumis melo)*

Resistance to CMV was first reported to occur in oriental melons, particularly in the pickling types *(C. melo* var. *conomon)*[9]. One study, involving the cultivar Freeman's Cucumber, indicated that resistance was conditioned by 3 recessive genes[21]. A second study with two Japanese cultivars revealed that resistance was due to 2 or 3 complementary recessive genes[58]. In France, using a melon from Korea, it was determined that resistance was oligogenic and recessive[48]. Additional research clearly indicated that resistance is strain specific[26]. Resistance in most oriental melons is strongly influenced by temperature. It was reported that at high temperatures (28°C) inoculated plants were symptomless and there was a limited viral replication. Conversely, at lower temperatures (19°C) plants developed symptoms[59]. Hence, this resistance is very suitable for warm areas of the world, such as the tropics.

Squash *(Cucurbita* spp.)

Tolerance to CMV, reportedly conferred by two recessive genes, was found in *C. pepo* (PI 176959 and PI 174192, Turkey)[31]. However, this tolerance has not been used because it is strain specific. A high level of resistance was located in

Table 1 Viruses and plant breeding

1. Resistance can take a range of forms: hypersensitivity, tolerance, resistance to virus spread, extreme resistance or immunity.
2. Most of the single major genes for resistance have proved to be rather stable.
3. In several cases, resistance is viral-strain-specific, One gene for each specific strain.
4. Resistance controlled by multiple gene systems is usually difficult to transfer.
5. New viral strains can arise from point mutations, deletions, or recombinations of existing strains.
6. Classification of virus strains based upon a range of characters tend not to coincide with grouping based upon ability to be controlled by specific host genes.
7. Only resistance genes can effectively differentiate these strains *(Pathotypes).* In most cases, serology cannot be used for this purpose, since serotypes and pathotypes rarely coincide.
8. Resistance-breaking strains of viruses usually have biological defects that may prevent them from becoming prevalent.
9. It is desirable to be alert to possible changes in prevalence and severity of viral diseases when new crops or cultivars are introduced in a given area.
10. More information is needed regarding crop losses caused by viruses, but reliable and practicable surveys are usually difficult to design and conduct.

Table 2 Viruses affecting cucurbits

TRANSMITTED BY APHIDS
- Cucumber mosaic (CMV)
- Clover yellow vein (CYVV)
- Muskmelon vein necrosis (MVNV)
- Watermelon mosaic 1 (WMV-1)
- Watermelon mosaic 2 (WMV-2)
- Zucchini yellow fleck (ZYFV)
- Zucchini yellow mosaic (ZYMV)

TRANSMITTED BY BEETLES
- Squash mosaic (SqMV)

TRANSMITTED BY FUNGI
- Cucumber necrosis (CNV)
- Melon necrotic leafspot (MNLSV)

TRANSMITTED BY LEAFHOPPERS
- Beet curly top (BCTV)

TRANSMITTED BY NEMATODES
- Tobacco ringspot (TRSV)
- Tomato ringspot (TmRSV)

TRANSMITTED BY THRIPS
- Tomato spotted wilt (TSWV)

TRANSMITTED BY WHITEFLIES
- Squash leaf curl (SLCV)
- Melon leaf curl (MLCV)
- Cucumber vein yellowing (CVYV)
- Cucumber yellows (CYV)

TRANSMITTED BY UNKNOWN VECTORS
- Cucumber green mottle mosaic (CGMMV)
- Cucumber pale fruit viroid (CPFV)

Table 3 Resistance to viruses in *Lagenaria siceraria*

Virus	Year	Author	Sources of resistance
CMV	1978	Greber	One Australian cultivar
	1981	Provvidenti	PI 269506 (Pakistan), PI 271353 (India), PI 391602 (China)
SqMV	1981	Provvidenti	All the accessions are resistant
WMV-1	1981	Provvidenti	PI 188809 (Philippines), PI 271353 and PI 288499 (India), PI 280631 (S. Africa), PI 391602 (China), and cv. Hyotan (Hawaii)
WMV-2	1981	Provvidenti	PI 271353 (India), PI 391602 (China), and cv. Hyotan (Hawaii)
ZYMV	1984	Provvidenti *et al.*	PI 271353 (India)

Table 4 Resistance to viruses in *Benincasa hispida*

Virus	Year	Author	Sources of resistance
WMV-1	1977	Provvidenti	PI 391544 and PI 391545 (China)
WMV-2	1977	Provvidenti	Tolerance in PI 391544 and PI 391545 (China)

wild species (*C. cordata, C. cylindrata, C. digitata, C. ecuadorensis, C. ficifolia, C. foetidissima, C. gracilior, C. lundelliana, C. martinezii, C. okeechobeensis, C. palmata, C. palmeri,* and *C. pedatifolia*)[44]. Using interspecific crosses, resistance from *C. martinezii* has been transferred into *C. pepo*[66], in which it appears to be partially dominant. Recently, good sources of resistance have been found in a few accessions of *C. moschata* and *C. maxima* from South America (unpublished).

Watermelon (*Citrullus lanatus* and *Citrullus* spp.)

Species of the genus *Citrullus* usually respond to CMV inoculation with only a localized infection, hence they are considered to be resistant. However, there is a specific strain of CMV which is able to infect them, causing prominant foliar and fruit symptoms[22]. This strain appears be of rare occurrence.

Bottlegourd (*Lagenaria siceraria*)

Resistance was reported in an Australian cultivar[14], and in PI 269506 (Pakistan), PI 271353 (India), and PI 391602 (China)[40]. No information is avialable regarding the inheritance of resistance.

Watermelon Mosaic Virus I (WMV-1)

A potyvirus with flexuous rods about 750 nm containing a single strand of RNA, it is spread by 19 aphid species in a stylet-borne manner and is easily transmitted mechanically, but is not seed-borne. WMV-1 natural host range is confined to Cucurbitaceae, but experimentally can infect locally *Chenopodium* spp. This virus is of common occurrence in the tropics, where it is often very destructive. Occasionally, it occurs also in temperate zones. Foliage of infected plants are severely affected by mosaic, distortion, and show very narrow lamina. Fruits are malformed, exhibiting color break and knobby overgrowths. WMV-1 is closely related to papaya ringspot virus (PRSV), consequently, in the future it will be known as a strain (pathotype) of this virus (PRSV-W). WMV-1 is not able to infect papaya[17,47,62,73].

Resistance:

Cucumber (*Cucumis sativus*)

Resistance and tolerance were found in a few cultivars from Asia, South America, and Hawaii. Inheritance studies using the cultivar Surinam (Suriname) indicated that resistance is conditioned by a single recessive gene (*wmv-1-1*)[65]. This cultivar responds to infection with mild systemic symptoms usually confined to 1 or 2 leaves. New growth and fruits are free of symptoms[65]. A number of oriental cucumbers respond also with a mild, but persistent mottle. However, their fruits may be affected.

Melon (*Cucumis melo* and other *Cucumis* spp.)

A very high level of resistance was found in PI 180280 from India[71]. In a breeding line deriving from it, the resistance was established to be monogenically dominant (*Wmv-1*)[70]. A second allele at the same locus (*Wmv-1*^{1}*) was located in another Indian melon (PI 180283)*[50]. However, the second gene has no practical value, since it conditions a lethal necrotic hypersensitivity[36]. A very high level of resistance to WMV-1 was also found in an accession of *C. metuliferus* from South Africa (PI 292190)[42] in which resistance also is monogenically dominant (*Wmv*)[43]. Probably this gene is identical to that found in *C. melo*. Resistance to WMV-1 in melon seems to be very stable and not strain specific.

Squash (*Cucurbita* spp.)

Cucurbita ecuadorensis, C. ficifolia, and *C. foetidissima* were found to possess a high level of resistance[44]. Recently, tolerance was located in a *C. maxima* from Uruguay and resistance in a *C. moschata* from Nigeria[41,46].

Watermelon (*Citrullus lanatus* and other *Citrullus* spp.)

A recent report has indicated that resistance may be available in PI 179662 and PI 179878 (India) and PI 295848 (South Africa)[33]. Egusi, an accession of *C. colocynthis*, appears to be tolerant[33].

Table 5 Resistance to viruses in cucurbita species

VIRUS	YEAR	AUTHOR	SOURCES AND GENETICS OF RESISTANCE
CMV	1960	Martin	*C. peop*, 2 recessive, strain specific
	1978	Provvidenti *et al.*	*C. Ecuadorensis, C. martinezii* and 11 other wild species
	1985	Provvidenti	*C. maxima* (Argentina), *C. moschata* (Nigeria)
SLCV	1984	Mc Creight	Some tolerance in *C. pepo, C. moschata*
SqMV	1978	Provvidenti *et al.*	*C. ecuadorensis, C. Martinezii, C. okeechobeensis.*
WMV-1	1978	Provvidenti *et al.*	*C. ecuadorensis, C. ficifolia, C. foetidissima*
	1982	Provvidenti	Tolerance in *C. maxima:* Zapallito Redondo (Uruguay)
	1984	Provvidenti *et al.*	*C. moschata* (Nigeria)
WMV-2	1978	Provvidenti *et al.*	*C. ecuadorensis, C. ficifolia, C. foetidissima, C. pedatifolia.* Tolerant: *C. gracilior* and *C. sororia*
	1982	Provvidenti	*C. maxima* (China)
	1984	Provvidenti *et al.*	*C. moschata* (Nigeria)
ZYMV	1981	Lecoq et al	*C. ecuadorensis*
	1984	Provvidenti *et al.*	*C ecuadorensis, C. moschata* (Nigeria)

Table 6 Resistance to viruses in *Cucumis sativus*

VIRUS	YEAR	AUTHOR	SOURCES AND GENETICS OF RESISTANCE
CMV	1928	Porter	Chinese Long, Tokyo Long Green
	1942	Shiffries *et al.*	3 dominant genes and modifiers
	1961	Wasuwat & Walker	Single dominant (*Cmv*)
	1969	Kooistra	3 partially dominant
SqMV			Most cultivars are resistant
WMV-1	1984	Wang & Provvidenti	In Surinam (Suriname), single recessive (*Wmv*-1-1)
WMV-2	1971	Cohen *et al.*	In Kyoto 3-ft, single dominant (*Wmv*)
ZYMV	1985	Provvidenti	In TMG-I, single recessive (*Zym*)

Bottlegourd (*Lagenaria siceraria*)

PI 188809 (Philippines), PI 271353 and PI 288499 (India), PI 280631 (South Africa), PI 391602 (China), and the Hawaiian cultivar Hyotan are resistant[40].

Waxgourd (*Benincasa hispida*)

PI 391544 and PI 391545, both from China, are resistant[39].

Watermelon Mosaic Virus 2 (WMV-2)

A potyvirus with filamentous rods about 750 nm, containing a single strand of RNA, it is spread by 20 or more aphid species in a stylet-borne manner and is easily transmitted by mechanical means, but not through seeds. WMV-2 natural host range includes most of the Cucurbitaceae and many leguminous species, thus it can be easily found in the tropics, as well as in temperate regions. Generally, the symptoms caused by this virus are less severe than those incited by WMV-I, however, they may vary considerably, depending upon the species and viral strain involved. Symptoms include green mosaic, leaf rugosity, green veinbanding, and chlorotic ringspots. Fruits are not distorted, but some colors are adversely affected. Although this virus shares the same name with WMV-1, they are distinct entities. Eventually, WMV-2 will be known as WMV[17,47,62,73].

Resistance:

Cucumber (*Cucumis sativus*)

Many of the oriental cucumbers seem to be resistant or tolerant to WMV-2. A study has established that resistance in the Japanese cultivar Kyoto 3-feet is governed by a single dominant gene (*Wmv*)[3,49].

Melon (*Cucumis melo*)

No adequate level of resistance has been found to control this virus. Some cultivars seem to possess a low level of tolerance, which is strain specific (unpublished).

Squash (*Cucurbita* spp.)

The wild species *C. ecuadorensis, C. ficifolia, C. foetidissima,* and *C. pedatifolia* were reported to be highly resistant[44]. Tolerance was detected in *C. gracilior* and *C. sororia*[44]. A selection from a a Chinese cultivar of *C. maxima* (PI 419081) possesses a good level of resistance[41].

Watermelon (*Citrullus lanatus* and other *Citrullus* spp.)

Line WMR-4, a selection from the Nigerian cultivar Egusi (*C. colocynthis*) is highly resistant[69]. Tolerance and resistance were recently reported in several other lines, including PI 182934, PI 295848, PI 381740[33].

Bottlegourd (*Langenaria siceraria*)

PI 271353 (India), PI 391602 (China), and the cultivar Hyotan from Hawaii are resistant[40].

Loofah Gourd (*Luffa acutanqula*)

Resistance was found in one accession of this species[68].

Waxgourd (*Benincasa hispida*)

Tolerance was detected in PI 391544 and PI 391545, both from China[39].

Zucchini Yellow Mosaic Virus (ZYMV)

A recently recognized potyvirus (1981) with filamentous particles about 750 nm containing a single strand of RNA, it is efficiently transmitted by a number of aphid species in a stylet-borne manner. ZYMV is also easily transmitted by mechanical means, and although there is circumstantial evidence of seed transmission, it has been difficult to prove this avenue of spread. ZYMV was found almost simultaneously in Italy (squash) and in France (melon) and is now known to occur in 14 countries on 5 continents. This is one of the most destructive viruses occurring in cucurbits in the tropics as well as in temperate zones. The severe foliage and fruit symptoms incited by this virus strongly resemble those caused by WMV-1; thus, it is often difficult to differentiate the

Table 7 Resistance to viruses in *Cucumis melo* and other C. species

VIRUS	YEAR	AUTHOR	SOURCES AND GENETICS OF RESISTANCE
CMV	1943	Enzie	Oriental Pickling Melons (*C. melo* var. *conomon*)
	1975	Karchi *et al.*	Freeman's Cucumber, 3 recessive genes
	1977	Risser *et al.*	Korean melon, 2-3 recessive genes
	1977	Takada	Japanese melons, 2-3 recessive genes
OGMMV	1979	Kroon *et al.*	Several wild *Cucumis* species
	1982	Nijs, den	*C. anguria*, single dominant (*Com*)
SqMV			*C. melo*, no resistance is available
	1978	Provvidenti *et al.*	*C. metuliferus*, PI 292190 (S. Africa)
WMV-1	1962	Webb & Bohn	*C. melo*, PI 180280 (India)
	1977	Webb	PI 180280, single dominant (*Wmv-1*)
	1971	Quoit *et al.*	*C. melo*, PI 180283 (India)
	1983	Pitrat & Lecoq	PI 180823, single dominant (*Wmv-1*)
	1974	Provvidenti & Rob.	*C. metuliferus*, PI 292190 (S. Africa)
	1977	Provvidenti & Rob.	PI 292190, single dominant (*Wmv*)
WMV-2			*C. melo*, no resistance is available
ZYMV	1984	Pitrat & Lecoq	*C. melo*, PI 414723 (India), single dominant (*Zym*)
	1984	Provvidenti *et al.*	PI 414723, immune to most American strains
	1984	Lecoq & Pitrat	Resistance strain specific

Table 8 Resistance to viruses in *Citrullus lanatus* and other C. species

VIRUS	YEAR	AUTHOR	SOURCES OF RESISTANCE
CMV	1971	Komuro *et al.*	Most *C.* species are resistant. One specific strain can infect
SqMV			Most *C.* species are resistant
WMV-1	1984	Munger *et al.*	Several PI's appear resistant
WMV-2	1977	Webb	Egusi (*C. colocynthis*) from Nigeria
	1984	Munger *et al.*	Several PI's appear tolerant
ZYMV	1984	Provvidenti *et al.*	Egusi (*C. colocynthis*) from Nigeria

symptoms incited by these two viruses under field conditions. In the tropics, ZYMV is often associated with WMV-1. A few strains and pathotypes of ZYMV are already known, complicating breeding for resistance. ZYMV is serologically related to WMV-2 and bean yellow mosaic virus (BYMV)[17,25,29,46].

Resistance:

Cucumber (*Cucumis sativus*)

Resistance and tolerance have been found in oriental cucumbers. A single plant selection (TMG-1) of the cultivar Taichung Mou Gua (China) is presently used for breeding purposes[46]. Preliminary data indicate that resistance is monogenically recessive with one strain and partially dominant with another strain. However, since fruits produced by heterozygous plants react with symptoms to both strains of the virus, this gene should be considered fully recessive, for breeding purposes (unpublished).

Melon (*Cucumis* melo)

A high level of resistance was found in some of PI 414723 (India), which are also resistant to WMV-1[37]. Studies indicated that resistance is conditioned by a single dominant gene (*Zym*)[37]. However, there are isolates of ZYMV that are able to overcome this resistance[27], clearly indicating that it is strain (pathotype) specific.

Squash (*Cucurbita* spp.)

The multiresistant wild species *C. ecuadorensis* is resistant to all known strains of ZYMV[25,46]. An additional source of resistance is a Nigerian squash (*C. moschata*)[46]. Preliminary studies have indicated that in this latter species resistance is partially dominant (unpublished). No sources of resistance or tolerance were found in cultivars and land races of *C. pepo*, a species which is devastated by this virus[25,29,46].

Watermelon (*Citrullus lanatus* and *Citrullus* spp.)

Most of the cultivars of watermelon are very susceptible, however, a good source of resistance was located in the Nigerian cultivar Egusi, an accession of *C. colocynthis*[46]. Preliminary data indicate that resistance is inherited recessively and is strongly influenced by temperature. At 20°C. there is some systemic infection, whereas at 30° C. plants are highly resistant (unpublished).

Bottlegourd (*Langenaria siceraria*)

PI 271353 (India) is highly resistant[46]. This line is also resistant to CMV. SqMV, WMV-1 and WMV-2[40].

OTHER CUCURBIT VIRUSES TRANSMITTED BY APHIDS

Clover Yellow Vein Virus (CYVV)
(formerly the severe strain of BEAN YELLOW MOSAIC VIRUS)

A potyvirus[17] of common occurrence in legumes, in which it causes severe symptoms. In the northeastern USA, this virus is frequently found in yellow summer squash (*C. pepo*) causing numerous chlorotic leaf spots which tend to remain distinct[45]. However, the intensity of this leaf spotting is influenced by environmental factors. Fruits are not affected, but seed production is reduced[45]. CYVV was also found in squash grown in Italy[28]. Most of the *Cucurbita* spp. are resistant to this virus, however, a bush *C. maxima* (cv. Gold Nugget) proved to be susceptible[45].

Zucchini Yellow Fleck Yirus (ZYFV)

A potyvirus[17] that occurs in the Mediterranean area, particularly in squash (*C. pepo*) grown in Italy and Greece[63]. It incites foliar pin point yellow flecks in squash and fruit malformation in cucumber[64].

Muskmelon Vein Necrosis Virus (MVNV)

A virus with rod-shaped particles of about 674 nm long, that appears to be restricted to *Cucumis melo*, and its botanical varieties *reticulatus, inodorus,* and *chito* in which it induces a dis-

tinct veinal necrosis in all but the apical leaves of affected plants. Petioles also become necrotic, together with a necrotic cork-like streaking of the stems. This virus is able to infect numerous legume species and it is serologically related to red clover vein mosaic virus (RCVMV)[12].

VIRUSES TRANSMITTED BY BEETLES

Squash Mosaic Virus (SqMV)

A comovirus with isometric particles about 30 nm in diameter, in which the single strand of RNA is divided into two functional pieces (M-RNA and B-RNA), it is mainly transmitted by seed and efficiently spread by striped and spotted cucumber beetles (*Acalymma* spp. and *Diabrotica* spp.). These insects acquire the virus within 5 minutes and can retain it for about 20 days. SqMV does not multiply in the vector, but can be recovered from regurgitation fluid, feces, and hemolymph. In nature, the host range of this virus is limited to Cucurbitaceae, particularly squash and melon species, in which most of the infection can be traced to infected seeds. Two pathotypes have been characterized: a) Strain I causing severe symptoms in melon but mild in squash, and b) Strain II which causes severe symptoms in squash and mild in melon. These two pathotypes are also serotypes, thus they can be easily distinguished by using immunodiffusion tests. Plants infected with SqMV may show a variety of symptoms, including mosaic, ringspots, green veinbanding, and protrusion of veins at the foliar margin. Under certain environmental conditions, squash may develop prominent enations on the underside of infected leaves[2,34].

Resistance:

Cucumber (*Cucumis sativus*)

Although SqMV can infect cucmber and related species, symptoms are usually mild and there is no malformation of fruits, thus this virus is not of economic importance.

Melon (*Cucumis melo* and *Cucumis* spp.)

No resistance has been found to either strain of this virus. However, some land races and botanical varieties of *C. melo* are tolerant to the squash strain, but not to the melon strain. Resistance was reported in *C. metuliferus,* which responds only with chlorotic local lesions, making this species a valuable assay host[42].

Squash (*Cucurbita* spp.)

Tolerance was reported in the wild species *C. ecuadorensis, C. martinezii,* and *E., okeechobeensis*[44].

Watermelon (*Citrullus lanatus* and *Citrullus* spp.)

SqMV is not of economic importance in these species.

Bottlegourd (*Langenaria siceraria*)

All the accessions of this species were found to react only with small and distinct necrotic local lesions without systemic infection, thus they are considered as highly resistant[40]. Since some plants tend to respond with numerous lesions, this hypersensitivity can be exploited for quantitiative and qualitative assays.

VIRUSES TRANSMITTED BY LEAFHOPPERS

Beet Curly Top Virus (BCTV)

A geminivirus with isometric particles about 20 nm each, occurring singly or in pairs, containing a single strand of RNA, this virus is restricted to the phloem and it is transmitted mainly by the leafhopper *Circulifer* spp., in which it circulates without multiplying. BCTV possesses a wide host range causing yellows-type diseases and a prominent leaf curling and distortion. Cucurbit plants are generally severely stunted, showing upward rolling of the leaf laminae and rosetting of apical growth. Fruits are malformed. This virus occurs in the arid and semiarid regions of America and Eastern Mediterranean Basin where it apparently originated[60]. No information is available regarding sources of resistance.

VIRUSES TRANSMITTED BY NEMATODES

Tobacco ringspot virus (TRSV) and tomato ringspot virus (TmRSV) belong to the nepovirus group, with particles about 28 nm containing two pieces of RNA (RNA-1 and RNA-2), which are essential for replication and pathogenicity. These viruses are mechanically, seed, pollen, and nematode transmitted. The nematodes *Xpihinema* spp. and *Longidures* spp., after acquiring these two viruses, can retain and spread them for months. However, these viruses do not multiply in the vectors nor are they transmitted though eggs. Virus particles are associated with specific sites of the alimentary tract, such as the stylet lumen or guiding sheath, or in the esophagus. TRSV and TmRSV are distinct entities and not serologically related, but they share a large host range inciting similar symptoms[15,54,55].

Tobacco Ringspot Virus (TRSV)

Cucumber and melon are particularly affected by this virus. Newly infected leaves usually exhibit a very bright yellow mosaic (acute phase). Although subsequent growth shows less prominent symptoms leaf size and internode length are considerably reduced (chronic phase). Fruits tend to abort or remain small and mottled. In squash infected with TRSV, symptoms are usually mild and transitory, but the virus is still present in symptomless growth. TRSV is seed transmitted in *Cucumis melo*. Resistance was found in several *Cucurbita* spp.[44] and in some accessions of *Cucumis anguria* (unpublished). No resistance has been reported in *C. sativus, C. melo*, or *Citrullus* spp.

Tomato Ringspot Virus (TmRSV)

This virus is able to cause severe symptoms in summer and winter squash (*C. pepo. C. mazima, C. moschata*, and *C. mixta*), but it causes only mild and transitory symptoms in cucumber melon, watermelon and other cucurbit species. Resistance is of common occurrence in wild squash. *C. cylindrata, C. digitata, C. ecuadorensis, C. gracilior, C. palmata, C. palmeri*, and *C. sororia*[44]. The following accessions of *Lagenaria siceraria* are also resistant: PI 188809 (Philippines) and PI 271353 (India)[40].

VIRUSES TRANSMITTED BY THRIPS

Tomato Spotted Wilt Virus (TSWV)

An RNA containing virus with membrane bound isometric particles about 70-90 nm in diameter, it is spread by *Thrips* spp. and Frankliniella spp. and is also transmitted mechanically. TSWV occurs mostly in the tropics where it infects a large number of plant species, which usually respond with chlorosis, necrosis, stem streak, and wilting[18].

Resistance:

Most of the cucurbit species respond only with local infection, thus they can be considered to be resistant. However, recently a silver mottle disease of watermelon was found to be caused by this virus[19].

VIRUSES TRANSMITTED BY WHITEFLIES

Squash Leaf Curl Virus (SLCV)

A recently recognized geminivirus (1981) with particles about 22x38nm containing a single strand of RNA. It is spread by the whitefly *Bemisia tabaci* in which it is circulative with a relatively long latent period. This virus can be transmitted mechanically, provided that an adequate buffer is used. In squash, it causes reduction of leaf size, thickening of veins, upward curling, enations and plant stunting. Serologically, it is related to bean golden mosaic virus and cassava latent virus. SLCV has been found in Southern California and Mexico, where it causes

severe economic losses[5,10].

Resistance:

Tolerance was reported in some cultivars of *C. moschata* and *C. pepo*[32], but whether it can be exploited remains to be seen. Apparently, this virus is not able to infect cucumber, melon, or watermelon[5].

Melon Leaf Curl Virus (MLCV)

Virus particles appear to be identical to those of squash leaf curl virus from which, however, it differs serologically. This virus affects melon, watermelon, squash, cucumber, and bean and it is spread by *Bemisia tabaci* as well as mechanically[12].

Cucumber Vein Yellowing Virus (CVYV)

As a rod-shaped virus with long particles about 740-800nm consisting of a double-stranded DNA, it is therefore, unique among plant viruses, since the other DNA-containing plant viruses exhibit spherical symmetry[53]. CYVV is transmitted by *Bemisia tabaci* and also mechanically. However, experimentally, both methods of transmission appear to be inefficient. CVYV can infect many cucurbit species.

Species and symptoms range from chlorotic or necrotic dots to severe vein yellowing and stunting. Originally it was found in Israel and named bottlegourd mosaic virus[4]. No sources of resistance are known.

Cucumber Yellows Virus (CuYV)

Probably a closterovirus with long flexuous particles about 1000 nm, mostly confined to the phloem, it is transmitted by the whitefly *Trialeurodes vaporariorum*, but not mechanically[74]. It was originally reported from Japan[74] where it infected cucumber and melon. CuYV was also found to occur in melon in France[30]. Symptoms consist of interveinal chlorotic spotting along the veins. Eventually these spots enlarge and become golden yellow, but veins remain green. There is no major reduction in size of the main stem, but the number of lateral shoots is drastically reduced. The host range seems to be confined to Cucurbitaceae. No information is available regarding resistance.

VIRUSES TRANSMITTED BY FUNGI

Cucumber Necrosis Virus (CNY)

A virus with isometric particles about 31 nm in diameter containing a single strand of RNA, it is soil-borne and transmitted by the zoospores of the chytrid fungus *Olpidium cucurbitacearum*, and also mechanically. In nature, CNV is usually found only in greenhouse cucumbers, but experimentally can infect a wide range of plants. Symptoms consist of chlorotic spots with pinpoint necrotic centers, which usually fall out, leaving shot-holes of various sizes. Leaves may be severely deformed, occasionally showing dark green enations. Fruits remain small and green mottled, and plants are stunted. During the summer, symptoms are mild or indistinct. No information is available regarding sources of resistance[6].

VIRUSES WITH UNKNOWN BIOLOGICAL VECTORS

Cucumber Green Mottle Mosaic Virus (CGMMV)

A tobamovirus with rod shaped particles about 300 nm. containing a single strand of RNA, it is easily transmitted by foliage contact, soil contamination, and through seed. No biological vector is known. CGMMV has a restricted host range, involving mainly cultivated cucurbits. Several strains have been reported which can cause a variety of symptoms ranging from a mild foliar mottle to a very prominent bright yellow mosaic. Leaves and fruits may be distorted and reduced in size. The watermelon strain can induce internal

discoloration and decomposition of fruits. This virus has been reported from Europe and Asia, particularly in green house grown plants[16,22].

Resistance:

No resistance has been located in cucumber and related species, but several Asian cucumber cultivars remain symptomless following viral infection. Unfortunately, these cannot be considered as tolerant, because the reduction in yield is similar to that of known susceptible cultivars. Several *Cucumis* species were proved to be resistant[24]. In crosses between the CGMMV-resistant *C. anguria* with the susceptible *C. myriocarpus*, it was established that resistance is conferred by a monogenically dominant factor (*Com*)[35].

Cucumber Pale Fruit Viroid (CPFV)

This viroid has been reported from the Netherlands[61] and Japan[51]. Comparative studies have shown that CPFV, in its biological proprieties, to be identical to hop stunt viroid[52]. It has been found in cucumber grown under greenhouse conditions and the main characteristics of the disease are pale green fruits and crumpled flowers, with foliar rugosity and chlorosis. In melon, watermelon, and waxgourd the disease appears to be more severe. These plants are stunted with a bushy appearance and eventually die prematurely. CPFV is more common in the spring planting, and can be transmitted with sap during pruning and grafting. The host range includes many Cucurbit species[61].

Resistance:

It was reported that one Japanese watermelon and a cultivar of *Cucurbita moschata* were not infected by this viroid[51]. A *Momordica* spp. was also not infected by CPFV[61].

FUTURE REQUIREMENTS

Systematic surveys should be made in the major cucurbit growing areas of the world in order to monitor the occurrence of new viral diseases. Because of increased knowlege of plant viruses and more effective international cooperation among researchers, it is now possible to accomplish more in less time. For example ZYMV was unknown 5 years ago, but now it is recognized as one of the most destructive and widespread agents occurring in cucurbits[25,29,46]

Excellent results have been obtained in breeding for resistance to diseases of cucurbits[57]. However, efforts must continue to locate sources of resistance in those species for which no resistant germplasm is presently available. For example, little is known regarding resistance to viruses occurring in *Benincasa, Luffa, Momordica, Sechium,* and *Trichosanthes* that are extensively cultivated in the tropics. With concentrated efforts results can be rapid and rewarding. Once again ZYMV is an example. Because of research conducted in Geneva, NY (USA) and Montfavet (France), significant progress was made in locating sources of resistance to this virus in less than 3 years. Cucurbit breeders have now at their disposal factors for resistance in *Citrullus, Cucumis, Cucurbita,* and *Lagenaria*[25,29,46]. It is worthwhile to mention that all the sources of resistance are derived from plants of tropical origin.

In developing viral resistant cucurbit cultivars, it is advisable to incorporate resistance genes into well known local varieties. In so doing, the main horticultural characteristics of these lines should be preserved, assuring their acceptability by the local markets. Eventually, the same breeding programs will lead to new generations of high performance cultivars, which will replace the established varieties.

Finally, genes for resistance can be efficiently used for virus identification. Laboratories in developed countries are usually well equipped, which makes virus characterization a relatively simple task. Since facilities are very limited in most other countries, very specific tests can be accomplished using host plants possessing single genes for resistance. These tests are perhaps the most useful of those based upon biological

response, and in most cases, offer the only opportunity to identify viral pathotypes. Resistant plants are also useful in separating individual viruses from mixtures.

This review of the literature has clearly shown the need for more research on cucurbit viruses and additional sources of resistance. It has also demonstrated the value of international cooperation in solving problems affecting cucurbit crops.

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A STRAIN OF CUCUMBER GREEN MOTTLE MOSAIC VIRUS ON BOTTLEGOURD IN TAIWAN

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SUMMARY

Bottlegourd plants showing mosaic symptoms were collected from Taichung and Hualien in 1982. A rod-shaped, sap-transmissible virus was isolated. This virus was identified as cucumber green mottle mosaic virus (CGMMV) by electron microscopy and bioassay. Virus particles are straight rods at 300 nm in length and 18 nm in diameter. They form crystalline inclusions in the cytoplasm of infected cells. This is the first record of CGMMV in Taiwan. Unlike the seven known strains, our virus isolate caused small, chlorotic local lesions on Chenopodium amaranticolor, symptomless infection on the inoculated leaves of both Datura stramonium and Petunia hybrida, and no infection on other test plants. This isolate, therefore, is considered a new strain of CGMMV.

摘　要

1982年，自臺中及花蓮地區採得具有嵌紋症狀之扁蒲病株，由其病葉分離出一種可由汁液感染之桿狀病毒。病毒顆粒之長度爲 300 毫微米(nm)，寬度18毫微米，在被害細胞之細胞質內形成結晶狀內含體。本病毒經電子顯微鏡觀察及生物檢定之結果，鑑定爲胡瓜綠斑嵌紋病毒(Cucumber Green Mottle Mosaic Virus, CGMMV)。本病毒在臺灣爲首次發現。本病毒除在藜 *(Chenopodium amaranticolor)* 上產生局部斑點外，又可在曼陀羅 *(Datura stramonium)*及矮牽牛 *(Petunia hybrida)* 上引起無病徵感染。與國外已確認之(CGMMV)之7個系統比較，雖然與西瓜系統(Watermelon strain) 相似，但不感染西瓜，又與印度系統C(Indian strain C)類似，但在矮牽牛上引起無病徵感染，因此判定爲一新系統。

摘　要

1982年臺中および花蓮で採取したモザイク症状を持つヒョウタンからかん状の汁液傳染性のウイルスが分離され、電顕観察と生物検定によりcucumber green mottle mosaic virus と同定された。ウイルス粒子は直かん状で長さ300 nm 巾18 nm である。感染細胞の細胞質中に結晶性封入體を作る。本ウイルスの臺湾における發生のはじめての報告である。既知7系統とは異なり本ウイルスは *Chenopodium amaranticolor* に小さな chlorotic local lesion を作り、*Datura stramonium* および *Petunia hybrida* の接種葉に無病徵感染し、他の供試植物には感染しなかつた。それ故この分離株はCGMMVの新系統と思はれる。

A STRAIN OF CUCUMBER GREEN MOTTLE MOSAIC VIRUS ON BOTTLEGOURD IN TAIWAN

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INTRODUCTION

Bottlegourd (*Lagenaria scieraria* Standl) is a popular cucurbitaceous crop in Taiwan. Some bottlegourd plants showing mosaic symptoms were collected from Taichung and Hualien in August, 1982. A rod-shaped, sap-transmissible virus was isolated and identified as cucumber green mottle mosaic virus (CGMMV). This virus is a member of tobamovirus[3,11] and was first described as cucumber virus 3[1]. It infects cucumber, watermelon, bottlegourd and muskmelon and causes severe yield loss of these cucurbitaceous crops in Japan, India, and Europe[3,5,9,11]. Seven strains of the virus have been recognized; most of them are hosted in cucurbitaceae[3,9]. East European isolate of the aucuba mosaic strain is an exception among them which infects tobacco cvs Samsun and Xanthi-nc[2]. The virus strains can be distinguished by differential hosts such as *Chenopodium amaranticolor, Datura stramonium, Petunia hybrida*, and other indicator plants[3,5,11]. Attempts were made to determine the strain characteristics of our virus isolate by electron microscopy and bioassays.

MATERIALS AND METHODS

Virus sources: Diseased plants were collected from Taichung and Hualien in August 1982. The virus isolate was obtained by single lesion isolation from a mechanically inoculated *C. amaranticolor.* It was then propagated on bottlegourd plant for the following experiments.

Electron microscopy: Leaf extracts were negatively stained with 2% phosphotungstic acid (pH7.0) and examined with JEM-7 electron microscope.

Healthy and virus-infected bottlegourd leaf pieces (1mm x 5mm) were fixed in 5% glutaraldehyde (in 0.1M phosphate buffer, pH7.0) at 4°C for two hr., and post-fixed in 1% osmium tetroxide at 4°C for another two hr. Following fixation, the tissue pieces were dehydrated in 2,2,-dimethoxypropane, and embedded in LX 112 epoxy resin[6]. Ultrathin sections were cut with a glass knife fixed on a Leitz Fernandez-Moran type ultramicrotome. Thin sections were then double-stained with uranyl acetate and lead tartrate[7] and examined with JEM-7 electron microscope.

Bioassays: One gram of diseased sample was ground with 10 ml of 0.1M phosphate buffer (pH7.0), then inoculated onto the leaves of the indicator plants *Chenopodium amaranticolor, C. murale, Datura stramonium, Petunia hybrida* and *Citrullus vulgaris.*

RESULTS

Sympromatology: Virus-infected bottlegourd leaves developed a well-defined mosaic symptom in the field. Blisters were occasionally found on dark green areas of infected leaves (Fig. 1). In addition, shortened internodes, stunting and smaller leaves were also apparent on the diseased plants.

Systemically infected bottlegourd leaves, which had showed a slight mosaic symptom

earlier, developed mottling symptom within two weeks after inoculation in the greenhouse (Fig. 2). In the later stage of disease development, a well-defined mosaic was formed and the size of leaves was significantly reduced. On an infected cucumber plant (*Cucumis sativus*), young leaves first turned dark green, which was then followed by vein-clearing. At the late stage, the size of leaves was also reduced (Fig. 3).

Electron microscopy: The negative stain of leaf extracts by PTA indicated that the virus is a straight rod with a normal length of 300 nm and a diameter of 18 nm (Fig. 5). Crystalline inclusions containing virus particles were found in the cytoplasm of infected cells (Fig. 6, 7). Accumulation of starch granules in chloroplasts was also common (Fig. 7).

Bioassays: This virus caused chlorotic local lesions with a diameter of 2-3 mm on inoculated leaves of *C. amaranticolor* (Fig. 4). It infected symptomlessly on inoculated leaves of *D. stramonium* and *P. hybrida*, on which the infection was determined by electron microscopic examination. However, the virus was not able to infect the other test plants, *C. murale* and *C. vulgaris*.

Fig. 1. Yellow and dark green blister mosaic symptoms on a bottlegourd leaf naturally infected by CGMMV

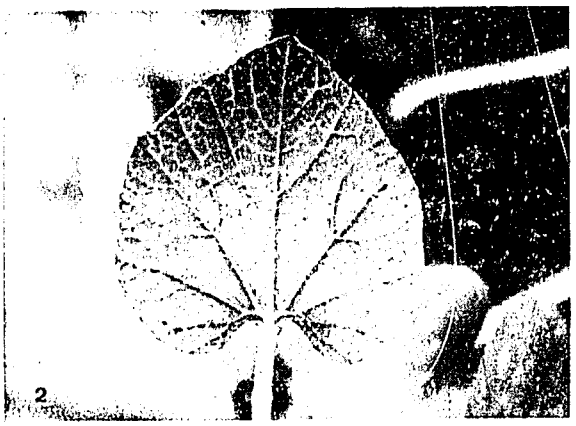

Fig. 2. Systemic mottling symptom on a young bottlegourd leaf 15 days after inoculation with CGMMV

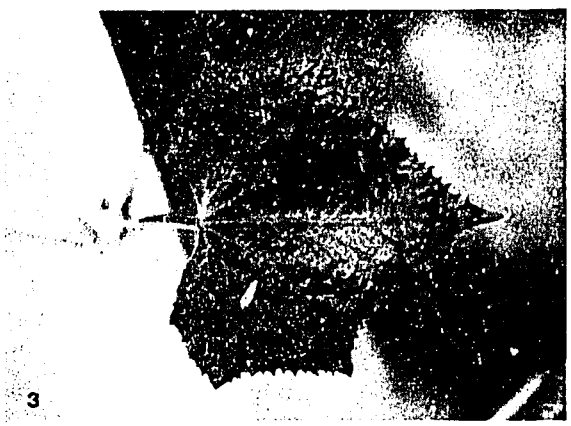

Fig. 3. Systemic darkgreen and vein-clearing symptoms on a cucumber leaf 15 days after inoculation with CGMMV from bottlegourd

Fig. 4. Chlorotic local lesions on a leaf of *Chenopodium amaranticolor* inoculated with CGMMV from bottlegourd

Fig. 5. CGMMV particles negatively stained with 2% phosphotungstic acid. Bar represents 500nm.

DISCUSSION

According to the results of bioassays and electron microscopy, this virus has been determined to be CGMMV. CGMMV was found only on cucurbitaceous crops. Several strains have been reported, and most of them possess restricted host ranges. The type strain reported in United Kingdom (UK) and Europe, causes leaf mottling, blistering and distortion with stunted growth on cucumber. The yield loss caused by this strain can be as much as 15%[3]. The aucuba mosaic strain reported in UK, Europe and India, causes bright yellow leaf mottling, slight leaf distortion and stunting of cucumber. Infected fruits develope yellow or silver-colored streaks and flecks, especially at high temperature[3]. The watermelon strain recorded in Japan, causes slight leaf mottling and dwarfing on watermelon, but its infection on fruits induces serious internal discoloration and decomposition of the fruits which are bitter to taste[3,4,5,10]. The muskmelon strain in Western Germany, has affected 70 to 80% of muskmelon plants in different years[9]. The Indian strain C from bottlegourd in India, causes blister-mottling, stunting and yield loss of the crop[3,4]. Consequently, the virus is an important factor in production of quality cucurbitaceous crops.

So far, seven strains of CGMMV have been recognized mainly according to the results of serology and differential hosts[1,2,3,8]. The type strain does not normally cause symptoms on cucumber fruits. It only produces a few local lesions in *C. amaranticolor* under certain conditions. It does not infect *D. stramonium* or *P. hybrida*[1,3]. The cucumber aucuba mosaic strain, which is the same as cucumber virus 4, induces striking symptoms on cucumber fruits, local lesions on *C. amaranticolor* and systemic mottling on *C. murale*. But it does not infect *D. stramonium*. East European isolates of this strain have been reported to produce chlorotic local lesions on tobacco cvs Samsun and Xanthi nc[2,3]. The watermelon strain infects watermelon and induces

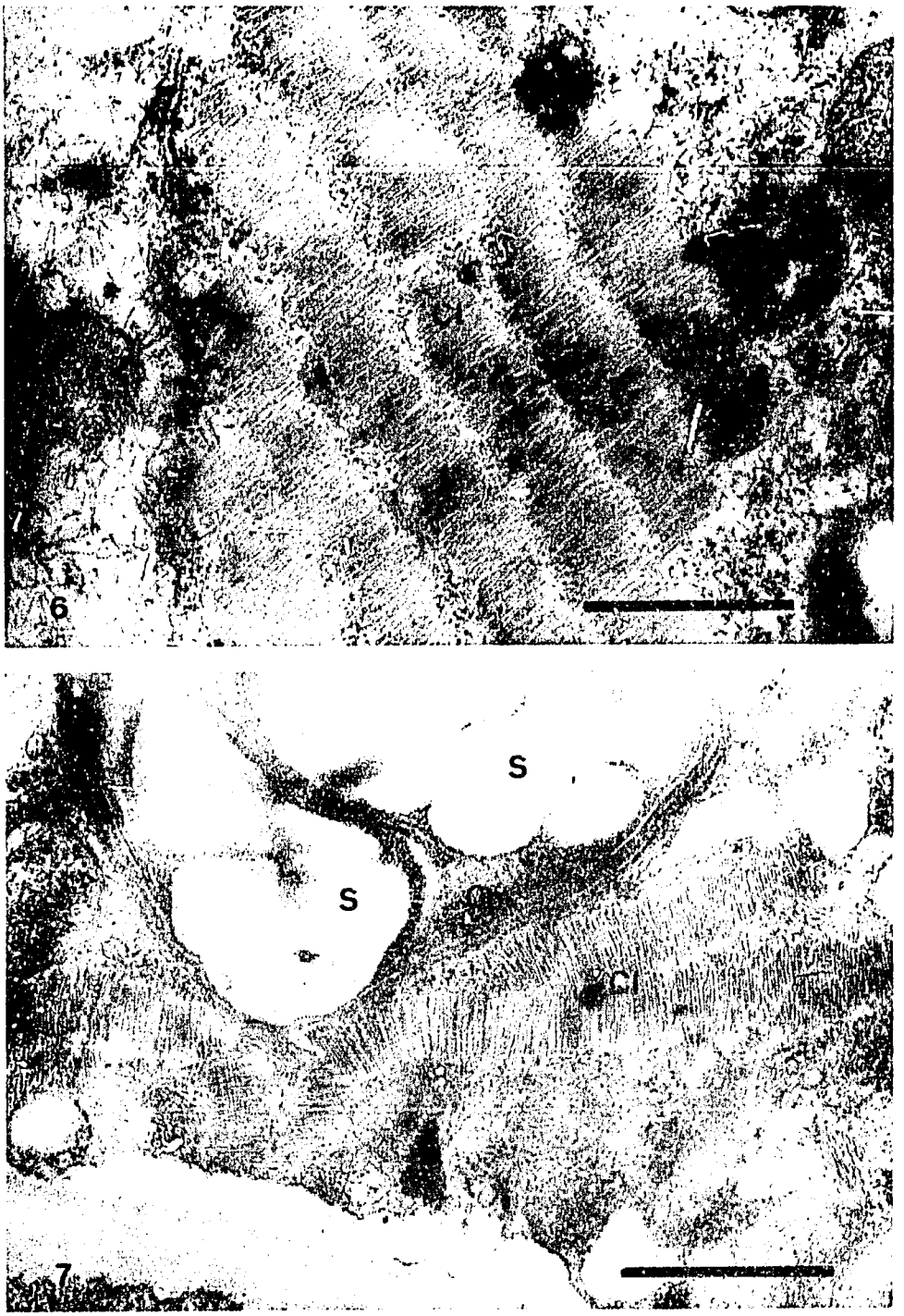

Fig. 6 & 7. Crystalline inclusions (CI) of CGMMV particles in cytoplasm and accumulation of starch granules (S) in chloroplast of infected cells of young bottlegourd leaves which showed mosaic symptoms 15 days after inoculation. Bar represents 1,000 nm.

local lesions on *C. amaranticolor*, but not on *D. stramonium*[3,4]. The Japanese cucumber strain causes severe fruit distortion on cucumber and induces local lesions on *D. stramonium*, but not on *C. amaranticolor*[3,4]. The Yodo strain from Japan causes fruit distortion on cucumber and local lesions on *C. amaranticolor*, *D. stramonium* and *P. hybrida*[3,5,10]. The Indian strain C causes local lesions on *C. amaranticolor*, symptomless infection on leaves of *D. stramonium* while no infection on tobacco or *P. hybrida*[3]. The muskmelon strain neither infects *C. amaranticolor* nor *D. stramonium*[9,11]. Unlike known strains, our isolates of CGMMV cause small, chlorotic local lesions on *C. amaranticolor*, symptomless infection on leaves of both *D. stramonium* and *P. hybrida*, but no infection on watermelon or *C. murale*. These properties show that our isolates are neither watermelon strain nor Indian strain C. This virus, therefore, is assumed to be a new strain of CGMMV.

This is the first report of CGMMV on cucurbitaceous crops in Taiwan. CGMMV can be transmitted through seeds, soil and mechanical means[3,4,5]. It is a limiting agent for growing bottlegourd, cucumber and probably other cucurbitaceous crops. Controls are needed to prevent the spread of CGMMV in Taiwan's cucurbitaceous crops.

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ZUCCHINI YELLOW MOSAIC VIRUS ISOLATE FROM CUCUMBER, *Cucumis sativus*: PURIFICATION AND SEROLOGY

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ABSTRACT

An isolate of zucchini yellow mosaic virus, ZYMV-7, was purified and an antiserum produced from leaves of inoculated zucchini plants taken 9-10 days after inoculation. Yields of purified virus were 18-22 mg/100 g of leaf tissues. A $_{260/280}$ from several separate preparations ranged from 1.27 to 1.31. The reciprocal titer of antiserum against homologous antigens was 4096 in ring interface precipitin tests, and 16 in SDS-immunodiffusion tests. Antiserum to ZYMV-7 produced precipitin bands with the eight other ZYMV isolates identified from Taiwan, and the ZYMV-444 isolate from Florida. Serological tests indicated that ZYMV isolates from Taiwan are different from the WMV-2 Florida isolate #486 but have some serological relations to certain types of WMV-2, while ZYMV-7 has no serological relationship to WMV-1 and WMV-2 (Florida isolate #486).

摘　要

矮南瓜葉片接種後 9～10 天會產生抗毒血清，並可純化矮南瓜黃化嵌紋病毒(ZYMV)。每百公克病葉可獲得20毫克之純化病毒。在數個樣品中，紫外光吸收比值A 260/280約在1.27至1.31 之間。利用界面環沉澱法將血清與純化病毒反應，血清力價爲4096，利用SDS 瓊脂免疫擴散法則爲16。

由本省之 8 個ZYMV分離株與佛大之ZYMV-444 均與 ZYMV-7抗血清形成沉澱帶。血清檢驗指出本省之 ZYMV 分離株與佛大 486 號植株分離之 WMV－2不同，但與WMV－2部分分離株有血清類緣關係，而ZYMV－7 對佛大 486 號分離株之WMV－1 與WMV－2 則無關。

摘　要

Zucchini yellow mosaic virus の 1 系統、ZYMV－7 を接種9～10日後のZucchini カボチヤの葉から純化し、抗血清を作製した。純化ウイルスの收量は病葉 100 g 當22mgで、260 nm/280nmの吸光度比は1.27～1.31 の範囲内にあつた。抗血清の力價は沉降反應で1/4096, SDS－ゲル内反應で1/16であつた。ZYMV－7抗血清は臺灣のZYMVの 8 分離株、フロリダのZYMV-444分離株と反應して沉降線を作つた。血清反應によれば台灣での分離株WMV－2のフロリダ分離株# 486 とは異なるが或種のWMV－2 と反應する。しかし、ZYMV-7 はWMV－1とWMV－2(Florida isolate # 486)とも反應しない。

ZUCCHINI YELLOW MOSAIC VIRUS ISOLATE FROM CUCUMBER, *Cucumis sativus:* PURIFICATION AND SEROLOGY

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INTRODUCTION

More than 25 viruses, including seven belonging to the potyvirus group, are found naturally in cucurbits.[16,22] Among them zucchini yellow mosaic virus (ZYMV) first described by Lisa *et al.* in Italy, 1981, is a relatively new potyvirus[15]. The virus has also been reported to occur in France[11], the United States[19,22] and Lebanon[13]. In Taiwan, cucumber mosaic virus (CMV)[4,27], watermelon mosaic virus type-1 (WMV-1)[12], cucumber green mottle mosaic virus (CGMMV)[26] and loofah mosaic virus (LoMV)[3] have been reported infecting cucurbitaceous plants. In 1982, we observed a disease which produced severe mosaic and rugose symptoms on leaves and distortion and malformation of fruits of cucumber plants. On the basis of symptoms, host range and serological relationships, we proposed that zucchini yellow mosaic virus was the cause of the disease[9]. Recently, in screening of 54 cucumber cultivars/lines for resistance to cucurbit viruses, Yang[27] found that ZYMV is the most destructive to cucumber crops.

Lisa *et al.*[15] had characterized and purified ZYMV. Recently, Provvidenti *et al.*[19] have also purified the cytoplasmic inclusion proteins from ZYMV infected plants. However, because of the tendency of the virus to aggregate, difficulties are often encountered in purifying the virus, especially for a high yield. In comparative studies, Huang Hseu[10] found that different ZYMV isolates greatly influenced the virus yield under same purification procedure, although these isolates appeared to be serologically identical in SDS-immunodiffusion tests[9]. This paper deals with a selected ZYMV isolate which we purified and consistently gave high yields of purified virus by modification to the procedure of Gonsalves and Ishii[8].

MATERIALS AND METHODS

Source and maintenance of the virus isolate

The ZYMV isolate used in this study referred to as ZYMV-7[9] was originally obtained from leaves of infected cucumber plants grown in the experimental field of Taiwan Agricultural Research Institute. It had passed through three serial transfers from a local lesion host, *Chenopodium amaranticolor*, to zucchini plants. The isolate produced severe mosaic and leaf distortion on zucchini, and latent infection on *Pisum sativum*, Alaska. It was generally maintained and propagated on zucchini plants throughout this study.

Determination of virus concentration in zucchini

To determine the time required for the virus to reach highest concentration in systemically infected leaves of zucchini plants, leaves were sampled from three inoculated plants at two day intervals. About six discs (0.5mm in diameter) were taken from each leaf by a cork borer. Then, the leaf discs were pooled, weighed, and ground in 0.05M potasium phosphate buffer, pH 7.5, with 1:10 ratio (w/v). The crude extract was inoculated on two *C. amaranticolor* plants to test for local lesions. The relative virus concentration in

systemically infected leaves was estimated by counting the number of lesions on lesion host 10-14 days after inoculation.

Purification

The purification procedure for ZYMV was a modification of that described by Gonsalves and Ishii[8] for purifying papaya ring-spot virus. Systemically infected leaves were harvested from greenhouse-grown zucchini plants 9-10 days after inoculation with ZYMV. The tissue was homogenized with a Waring blendor (Model 31BL42) in 0.25M potassium phosphate buffer (2ml/g tissue), containing 0.01M disodium ethylenediamine-tetraacetate (EDTA) and 0.1% sodium sulfite (Na_2SO_3), pH 7.5. Chloroform and carbon tetrachloride, each at 0.5ml/g tissue, were added slowly as the tissues were being ground, homogenate was centrifuged at 5,000 rpm for 5 min., and the supernatant centrifuged again at 8,000 rpm for 15 min. in a Hitachi RP-12 rotor. PEG (polyethylene glycol, MW. 6,000) was added to the supernatant at the rate of 8g/100ml. The mixture was stirred for 1-2 hr and was centrifuged at 10,000g for 20min. in a Hitachi RP-16 rotor. The resulting pellets were resuspended in 0.1M potassium phosphate buffer plus 0.01M EDTA, pH 7.5 and stirred for another hour. Before the second PEG treatment (5% PEG + 0.3M NaCl), the resuspended virus was spun at 4,000 – 5,000 rpm for 10 min. to remove the host constitutes. The virus was precipitated after second PEG treatment, and the pellets were finally resuspended in 0.05M potassium phosphate buffer, pH 7.5, and stirred overnight.

Upon isopycnic centrifugation, the virus suspension was mixed with 26% cesium sulfate (w/w), or of 30% cesium chloride. Centrifugation was performed at 38,000 rpm for 22-24 hr. at 6°C in a Hitachi RP-65T rotor ($w^2t = 1.28 \times 10^{12}$ rad^2/s). After centrifugation, the virus zone was collected by a small pipet. The purified virus suspension was diluted to five times its volume with phosphate buffer, and again centrifuged at 10,000g for 10min. The virus preparation was dialyzed overnight against 0.05M phosphate buffer to remove Cs salt for spectrophotometry, or it was given another cycle of isolpycnic centrifugation.

The purity and quantity of the purified virus preparations were determined by a Hitachi spectrophotometer (Model 220S). In one trial, the absorption profile was measured at 254nm with UV monitor (Gilson LC detector, Model 111) coupled with Gilson CRP fractionator for virus sample from the second cycle of isopycnic centrifugation in Cs_2SO_4.

Production of antiserum

All antisera to ZYMV-7 were produced in one rabbit by injecting virus purified after one cycle of isopycnic centrifugation. The rabbit was given a series of four intramuscular injections at weekly intervals, and a booster at six weeks after the final injection. The immunized rabbit was bled weekly, starting one week after the last injection. Antibodies reacting with host proteins were removed by adding 2% of bovine serum albumin (BSA) (Sigma Co.) to the sera, and usually incubating the mixture overnight at 4°C. All sera were stored at –20°C in the presence of 0.04% sodium azide.

Serology

The titer of antisera was determined either by ring interface precipitin test[2], and or by SDS-immunodiffusion test[14,20] against homologous virus. Serological reactions were made among the following ZYMV isolates: ZYMV-1 to ZYMV-9 from Taiwan[9], and ZYMV-444 from Florida. In addition, WMV-1 and WMV-2 (Florida isolate #486) were also included for the purpose of comparison. Both ZYMV-444 and WMV-2 were kindly provided by Dr. D. E. Purcifull, University of Florida. The agar medium for immunodiffusion tests consisted of 0.8% Noble agar, 1% sodium azide and 0.5% dodecyl sulfate sodium (SDS)[14,22], In some cases 2% bovine serum albumin was incorporated into the medium to remove the non specific reaction[4,20,23]. Crude antigens were

prepared from fresh leaves of inoculated zucchini plants by grinding 1ml/g tissue in distilled water, followed by adding 1ml of 3% SDS, and filtered through cheesecloth. Generally, the whole procedure was completed within one hour.

RESULTS

Virus concentration in zucchini

Zucchini leaf extracts prepared from inoculated systemically infected plants were assayed for virus concentration (content) by inoculation to *Chenopodium amaranticolor*. The local lesion produced on *C. amaranticolor* showed that virus content increased gradually in zucchini during the first six days, and then it increased rapidly. A peak virus concentration was reached in systemically infected plants about 8-10 days after inoculation under growth chamber conditions (25°C) (Fig. 1). Therefore, leaves were usually harvested at this time and used for virus purification and serological studies.

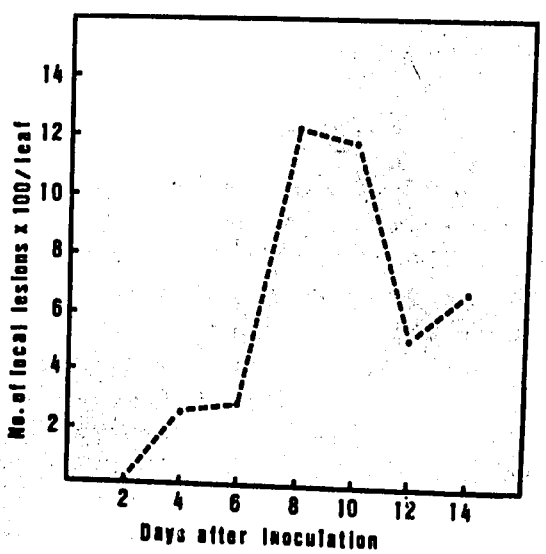

Fig. 1. Virus concentration of zucchini yellow mosaic virus in relation to the time of infection in zucchini plants under the constant temperature at 25°C, the relative virus conc. was estimated by counting the local lesions produced in *Chenopodium amaranticolor*.

Purification

In our earlier purification attempts, virus suspension was mixed with 30% Cs_2SO_4 for isopycnic centrifugation. The virus zone appeared at about 1.5cm below the meniscus after centrifugation for 22-24 hours using Hitachi RP-65T rotor tubes. It was close to the host green components and needed great care in collection by a small pipet. Later, the virus suspension was mixed with 26% Cs_2SO_4. After isopycnic run the virus zone was located just below the middle of the tube and was well separated from the green materials. The purified virus was easily collected from Cs salt gradient.

The purity and quantity of the purified virus preparations were analyzed by spectrophotometer. Virus recovered from the first isopycnic centrifugation was found contaminated with host proteins. However, a second cycle of isopycnic centrifugation yielded highly purified virus (Fig. 2). There were no significant differences in UV spectra of the purified virus preparations from one or two cycles of isopycnic contrifugation. The absorption of several separate preparations of the purified virus showed the min. at 246-247 nm and max. at 260 nm, and the O.D. ratio of $A_{260/280}$ ranged from 1.27 to 1.31 (uncorrected for light scattering). Assuming $E^{0.1\%}_{360} = 2.4$ for tobacco etch virus[21], yield of the purified virus was estimated to be 18-20mg/100g leaf tissues.

In one additonal trial for comparing virus stability in cesium sulfate and cesium chloride solutions, the virus suspension was mixed with Cs salts. After isopycnic centrifugation, virus preparations from both Cs salts were inoculated to *C. amaranticolor* after dialysis. At $O.D._{260}$ = 0.593, the purified virus from CsCl gradient produced 1835 lesions/leaf, and at $O.D._{260}$ = 0.528, that from Cs_2SO_4 gradient produced 1378 lesions/leaf. These results indicated that ZYMV-7 is stable in both Cs salts.

Serology

In ring interface precipitin tests the recipro-

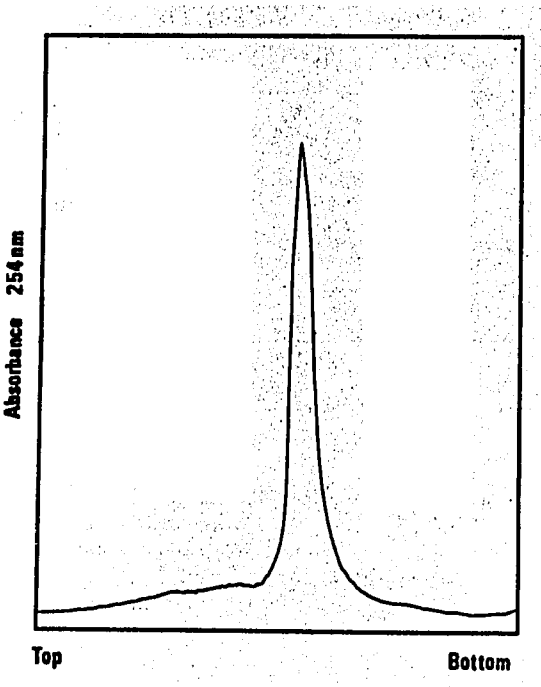

Fig. 2. Absorption profile of the purified zucchini yellow mosaic virus from the second cycle of isopycnic centrifugation in Cs_2SO_4.

cal titers of antiserum, taken from the third bleeding, against homologous antigen in crude leaf extracts and purified preparations were 2048 and 4096, respectively. This antiserum reacted with healthy plant extracts at 1:4 dilution. Reacting with crude antigen in SDS-immunodiffusion gel, the antiserum had a reciprocal titer of 16. There was no precipitin band formation when it was tested against healthy plant extracts. However, antisera collected from several bleedings reacted with host proteins weakly (Fig. 3,b). Generally, we added 2% BSA into the agar medium to remove the nonspecific reactions (Fig. 3,a).

ZYMV-1 to ZYMV-9, and ZYMV-444 appeared to be serologically identical in SDS-gel diffusion tests. Except that ZYMV-2 sometimes formed a weaker precipitin line, they formed strong precipitin bands without spur reaction when tested against antiserum to ZYMV-7 (Fig. 4,a,b) or antiserum to ZYMV-Italy (Fig. 4,c) (provided by Dr. V. Lisa). There were no serological relations between ZYMV-7 and WMV-1 and between ZYMV-7 and WMV-2 (Florida #486) (Fig. 3). However, an antiserum to WMV-2 obtained from Dr. D. E. Purcifull which reacted with WMV-2 Florida #486 strongly, also formed precipitin bands when tested against ZYMV-4, ZYMV-7 and ZYMV-444 (Fig. 4,d). This result indicated that ZYMV isolates from Taiwan were serologically related to certain types of WMV-2.

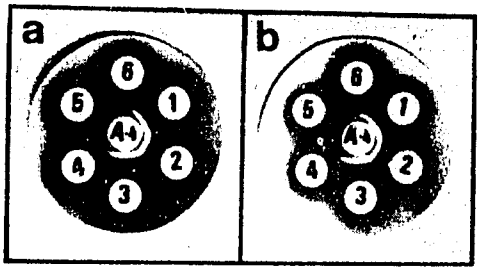

Fig. 3. Serological reactions of zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus-1 (WMV-1), and watermelon mosaic virus-2 (Florida #486) with antiserum to ZYMV-7.
The central wells (A-4) contained antiserum to ZYMV-7 collected 4 wks. after the last injection.
The peripheral wells contained SDS-treated antigens: 1: ZYMV-4; 2: ZYMV-444 (Florida isolate); 3: ZYMV-7; 4: WMV-2 (Florida #486); 5: WMV-1; 6: leaf extracts from healthy zucchini plants.
The medium for pattern (b) consisted of 0.8% Noble agar, 0.5% SDS, and 1% NaN_3, whereas medium for pattern (a) consisted of the above constitutents plus 2% BSA. The non-specific reaction was observed in (b), however, it disappeared in (a).

DISCUSSION

Aggregation of virus particles during purification has been a limiting factor in obtaining higher yields of purified virus in the PVY group[8,15,17,24,25]. ZYMV, a potyvirus recently reported by Lisa *et al.*[15], is no exception. The use of chloroform for clarification or of high molarity buffers for resuspension did not improve the yields of the virus[15]. In our initial purification attempts, an isolate designated ZYMV-4[9] was selected and the yield of purified virus was low, probably due to the losses of virus from aggregation during

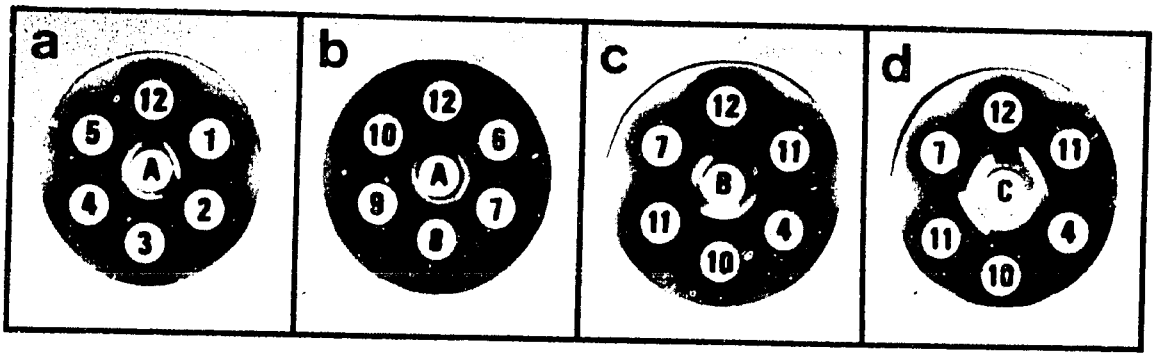

Fig. 4. Serological comparisons of zucchini yellow mosaic virus isolates and watermelon mosaic virus-2 (Florida #486) in SDS-immunodiffusion tests. The central wells (A) contained antiserum to ZYMV-7, (B) antiserum to ZYMV-Italy (kindly provided by Dr. V. Lisa), and (C) antiserum to WMV-2 (kindly provided by Dr. D. E. Purcifull). The peripheral wells contained SDS-treated antigens from zucchini leaves infected with: XYMV isolates Nos. 1-9 from Taiwan; 10 = ZYMV-444 (Florida isolate); 11 : WMV-2 (Florida #486); 12= healthy zucchini squash leaves.

purification; however, when ZYMV-7 was processed by the same procedure, quite a high yield of purified virus was obtained, suggesting that different ZYMV isolates greatly affect the yield of virus in purification[10].

The addition of EDTA to extraction or resuspending buffers to reduce virus aggregation had been proposed[5,8,24,25]. Deigado-Sanchez and Grogan[5] obtained an UV absorption spectrum typical of nucleoprotein for purified virus resuspended in borate buffer plus 0.01M EDTA. Our initial experiments showed that the additon of Na-EDTA at 0.01M in purified virus preparations changed the UV spectra. It also affected the value of O.D. $_{260/280}$. Therefore, while EDTA was used to prevent virus aggregation, its effect on spectrophotometric measurement had to be taken into account.

The purified virus prepared from either the first or the second isopycnic centrifugation gave an identical UV absorption curve; however, the virus preparations from one isopycnic centrifugation were still contaminated with host proteins as previously observed by others[1,8,24]. The value of $A_{260/280}$ ranging from 1.27 to 1.31 in our studies was a little high, presumably due to contamination with host nucleic acids by the use of phosphate buffer[6,21], but it fitted into the range of 1.2-1.37 reported for other potyviruses[7,18].

Our ZYMV isolates and the Florida isolate ZYMV-444 appeared to belong to the same serogroup in SDS-immunodiffusion tests. They were not serologically related to WMV-1 or WMV-2 (Florida #486). Lisa *et al.*[15] found that ZYMV reacted with two antisera against Italian isolates of WMV-2. Purcifull *et al.*[22] pointed out that antiserum to WMV-2 collected at late bleeding could react with ZYMV (Florida isolate 1119) antigen. In our studies, an antiserum to WMV-2, provided by Dr. D. E. Purcifull, reacted with our ZYMV isolates but formed spur reactions. This result confirmed that ZYMV isolates were serologically, but distinctly, related to certain isolates of WMV-2 as reported previously[15,22].

Since ZYMV has not been extensively investigated in Taiwan, the collection of more ZYMV isolates from different crops and study of their serological relationships to WMV-2 are necessary. ZYMV has been reported to be the most destructive virus to cucumber plants[27]; breeding programs designed to develop ZYMV-resistant varieties should evaluate cross progenies against a wide range of ZYMV isolates.

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VIRUS DISEASES OF SOLANACEOUS PLANTS TRANSMITTED BY WHITEFLY

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SUMMARY

The whitefly-borne eggplant yellow mosaic virus (EYMV) was transmitted by mechanical inoculation. Host range of EYMV was limited to seven plant species in the family Solanaceae. Host range of tomato leaf curl disease agent (Tm LCDA) was similar to that of tobacco leaf curl virus (TLCV). Each purified preparation from EYMV, TmLCDA or TLCV-infected plants consisted of geminate particles about 18 x 30 nm in size. The aggregates of virus-like particles were observed in the nuclei of phloem cells of leaves infected with EYMV, TmLCDA or TLCV.

摘　要

由粉蝨傳播的茄子黃色嵌紋病毒(EYMV)在機械傳播試驗中，測得之寄主，限於茄科之7種植物。番茄捲葉病毒(TmLCDA)之寄主範圍與菸草捲葉病毒(TLCV)相似。由EYMV，TmLCDA或TLCV感染之植株純化所得樣品，均發現含有18×30nm大小之geminate顆粒病毒。類似病毒之顆粒聚合體，亦可以在感染EYMV, TmLCDA或TLCV葉片之靱皮部細胞核內觀察到。

摘　要

コナジラミ傳搬性のeggplant yellow mosaic virus は汁液傳染する。本ウイルスの寄主範囲はナス科の7種の植物に限られる。tomato leaf curl病の病原の寄主範囲はtobacco leaf curl virus と同様である。以上3種のウイルスに感染した植物より純化した標本はジエミニウイルス（大きさ18×30nm)から成つている。3種ウイルスと感染葉のし部細胞の核の仁にウイルス様粒子の集塊が認められた。

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INTRODUCTION

In recent years, whitefly-borne virus diseases have become important and severe on bean, mungbean tomato, chilli and tobacco plants in various parts of the world and particularly in the tropics. During our studies on the identification of legumes virus diseases in Thailand, we detected whitefly-borne mungbean yellow mosaic virus, a member of geminivirus[3,14], and soybean crinkle leaf disease, the causal agent is possibly a member geminivirus[4]. We also observed eggplants with yellow mosaic, tomato plants with leaf curl and tobacco plants with leaf curl and enation symptoms. Preliminary studies in the laboratory revealed that the diseases were all from whitefly-transmissible agents.

In this paper, we report host range, transmission, purification and electron microscopy of whitefly-borne viruses of solanaceous plants and indicate that the causal agents may be a member of the geminiviruses.

MATERIALS AND METHODS

Virus sources and maintenance. Eggplants showing yellow mosaic, tomato plants showing yellow leaf curl and tobacco plants showing leaf curl symptoms were collected from fields in Nakhornpathom Province of central Thailand, Lampang Province of northern Thailand and Nongkhai Province of northeast Thailand, respectively. For each the causal agent of eggplant yellow mosaic disease (EYMD), tomato leaf curl disease (TmLCD) or tobacco leaf curl disease (TLCD) was isolated from eggplants, tomato and tobacco plants naturally infected by whitefly transmission and the agent then maintained in eggplants, tomato and tomato plants, respectively.

Mechanical transmission. Sources of the inocula were collected from systemically infected young leaves at 10-14 days after whitefly or grafting transmission. Then each inoculum was prepared by grinding young leaves in 0.1 M potassium phosphate buffer, pH 7.8, containing 0.1% thioglycolic acid. Inoculations were made by rubbing carborundum-dusted leaves of the test plants with a cotton swab soaked in the crude sap.

Seed transmission. Seed samples were harvested from EYMD-infected *Nicotiana tabacum* 'Xanthi nc' and *Datura metel.* Each group of 50 matured seeds were individually germinated in earthen pots containing steam-sterilized soil in an insect proof greenhouse. The percentage of seed transmission was determined by the symptoms on germinated plants.

Host range. Host range of the causal agents of EYMD, TmLCD and TLCD were determined by whitefly, *Bemisia tabaci* Genn., transmission. Acquisition and inoculation feeding periods ranged between 24 and 48 hr. The causal agent was transmitted by 10-15 viruliferous whiteflies to test plants. Host range of EYMD was also determined by grafting and mechanical transmission.

Stability in sap. In sap extracted from EYMD-infected *N. tabacum* 'Xanthi nc' leaves, thermal inactivation point (TIP), dilution end point (DEP) and longevity *in vitro* (LIV) of the causal agent were determined using *D. stramonium*

seedlings as test plants.

Virus purification. *N. tabacum* 'Xanthi nc' infected with the causal agent of EYMD and *Lycopersicon esculentum* infected with the causal agent of TmLCD or TLCD were used for virus purification. Systemically infected leaves were harvested 2-3 wk after transmission by grafting or mechanical inoculation. Healthy leaves were used as controls. Leaves were processed by previously reported purification methods of mungbean yellow mosaic virus[3]. Resuspended pellets after centrifugation in polyethylene glycol reverse concentration gradients and followed by ultracentrifugation, were used for electron microscopy, absorbance spectrum analysis and infectivity assays by mechanical inoculation to test plants.

Electron microscopy. Purified virus preparations for electron microscopy were mounted on collodion-carbon-coated grids and stained with 2% sodium phosphotungstate (PTA), pH 3.5, or 2% uranyl acetate.

Infected leaf samples for ultrathin sectioning were collected from inoculated plants 10-20 days after inoculation either by whitefly or by grafting. Pieces of the infected leaves were fixed with 4% glutaraldehyde at 5°C for 1.5 hr, and were postfixed with 2% osmium tetroxide in 0.1 M phosphate buffer, pH 7.5, at 5°C for 3-5 hr. After washing and dehydration, they were embedded in a mixture of low-viscosity epoxy resin[12]. Ultrathin sections were cut with a glass knife in a LKB 8800 Ultrotome. They were double-stained with uranyl acetate and lead citrate before observation. Leaf samples from noninoculated test plants at comparable age were similarly processed and served as controls. Observations were made with a Hitachi Model H300 or H500 electron microscope.

RESULTS

Symptomatology and host range. Among the 28 plant species belonging to five families used in whitefly transmission tests, only six species of the family Solanaceae were infected with the causal agent of EYMD (Table 1). In systemically infected leaves of eggplant (*Solanum melongena*) (Fig. 1A) and *D. metel*, small irregularly shaped chlorotic spots along the veinlets appeared at the early stage and developed into yellow mosaic symptoms. *Capsicum annuum*, *D. stramonium*, L. *esculentum* and *N. tabacum* 'White Burley' showed irregularly shaped chlorotic spots along the veinlets of infected leaves and young leaflets curled downward at the edges. Infected plants were usually stunted. *N. glutinosa* and *N. tabacum* 'Xanthi nc' were infected with the causal agent of EYMD by grafting and mechanical transmission but not by whitefly transmission.

Among the 28 plant species belonging to six families used in whitefly transmission tests, *Phaseolus vulgaris* 'Top Crop' of the family Leguminosae and *D. stramonium*, *L. esculentum*, *N. glutinosa* and *N. tabacum* of the family Solanaceae were infected with the causal agent of TmLCD (Table 1). Symptoms of the infected plants consisted mainly of leaf curling and stunting. Systemically infected leaflets of tomato became yellow at the edges and curled upward or downward (Fig. 1B).

Host range and symptomatology of the causal agent of TLCD were very similar to those of TmLCD (Table 1) except for appearance of enations on the lower surface leaves of *P. vulgaris* 'Top Crop', *N. glutinosa* and *N. tabacum* 'Xanthi nc' (Fig. 1C and D).

Mechanical transmission. The causal agent of EYMD could not be transmitted by mechanical inoculation in preliminary experiments, however, in later trials the agent could be transmitted using modified inoculation techniques of mungbean yellow mosaic virus[3]. The data of Table 2 shows that the causal agent of EYMD could be transmitted mechanically. *L. esculentum* and *N. tabacum* were relatively good sources of inoculum giving 50% and 100% infection, respectively. As test plants, *N. tabacum*, *D. stramonium*, *L. esculentum* and *D. metel* were relatively good, however, *S. melongena* were not susceptible (Table 2).

When around 20 adults of *B. tabaci* were left for 48 hr on mechanically infected *D. metel*, *D. stramonium* and *L. esculentum* and then transfered for 48 hr inoculation feeding on *D.*

Table 1. Host range of causal agent of eggplant yellow mosaic disease, tomato leaf curl disease or tobacco leaf curl disease (via whitefly)

Plants tested	Symptoms[1] EYMDA[2]	TmLCDA[3]	TLCDA[4]
Compositae			
Ageratum conyzoides	–	–	–
Eclipta prostata	–	–	–
Zinnia elegans	–	*	*
Cucurbitaceae			
Cucumis sativus	–	–	*
Leguminosae			
Arachis hypogaea	–	–	–
Cajanus cajan	–	–	–
Cassia tora	–	–	–
Dolichos lablab	–	–	–
Glycine max	–	–	–
Phaseolus anguralis	–	–	–
P. lunatus	–	–	–
P. vulgaris	–	LC, St	En, LC, St
Pisum sativum	–	–	–
Vicia faba	–	–	–
Vigna mungo	–	–	–
V. radiata	–	–	–
V. sesquipedalis	–	–	–
V. unguiculata	–	–	–
Malvaceae			
Abelmoschus esculentus	–	–	–
Malvastrum coromandelianum	–	–	–
Pedaliaceae			
Sesamum indicum	*	–	–
Solanaceae			
Capsicum annuum	ICS, LC, St	–	–
Datura metel	ICS, YM	–	–
D. stramonium	ICS, LC, St	ICS, LC	ICS, LC
Lycopersicon esculentum	ICS, LC, St	LC, St, Y	LC, St. Y
Nicotiana glutinosa	– (CS, LC, St)[5]	ICS, LC, St	En, ICS, LC, St
N. tabacum 'Xanthi nc'	– (ICS, LC)[5]	ICS, LC, St	En, ICS, LC, St
N. tabacum 'White Burley'	ICS, LC	ICS, LC, St	En, ICS, LC, St
Petunia hybrida	–	–	–
Solanum melongena	ICS, YM	–	–

1) Key to symptoms:
En = enation;
ICS = irregularly shaped chlorotic spot;
St = stunting;
YM = yellow mosaic;
* = not tested.
CS = chlorotic spot;
LC = leaf curling;
Y = yellowing;
- = no symptoms;

2) EYMDA = eggplant yellow mosaic disease agent.
3) TmLCDA = tomato leaf curl disease agent.
4) TLCDA = tobacco leaf curl disease agent.
5) Symptoms after grafting or mechanical transmission.

metel, D. stramonium, L. esculentum, N. tabacum and S. melongena, they could not transmit the causal agent to any of the five plant species. The causal agent of EYMD was transmitted from mechanically inoculated plants to healthy test plants by grafting. All attempts to transmit the causal agent of TmLCD or TLCD by mechanical inoculation failed.

Seed transmission. None of the 50 seedlings grown from seeds harvested from EYMD-infected *D. metel* or *N. tabacum* 'Xanthi nc' showed distinct symptoms.

Stability in sap. In sap extracted from EYMD-infected tobacco leaves, the causal agent showed TIP of 25°–30°C for 10 min, DEP between 10^{-1} and 10^{-2}, and LIV of 1 day at 20°C.

Virus purification. Partially purified preparations obtained from EYMD-infected tobacco leaves consisted of geminate particles about 18x30 nm in size (Fig. 2). The preparations with gemin-

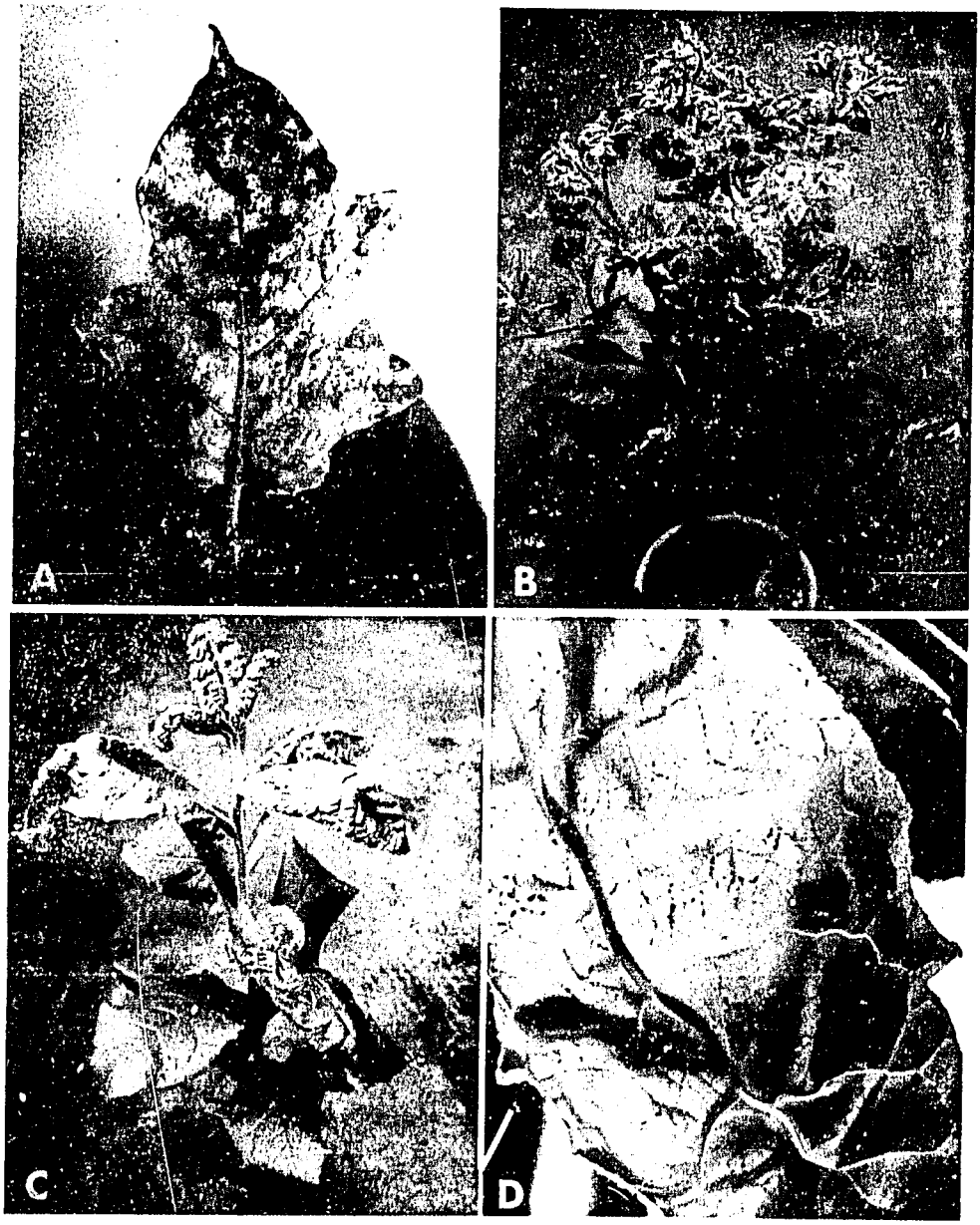

Fig. 1. Symptoms of infection with whitefly-borne viruses. (A) Yellow mosaic in eggplant. (B) Yellowing and leaf curling in tomato. (C and D) Severe downward curling and enations on the veinlets of the lower surface leaf of tobacco.

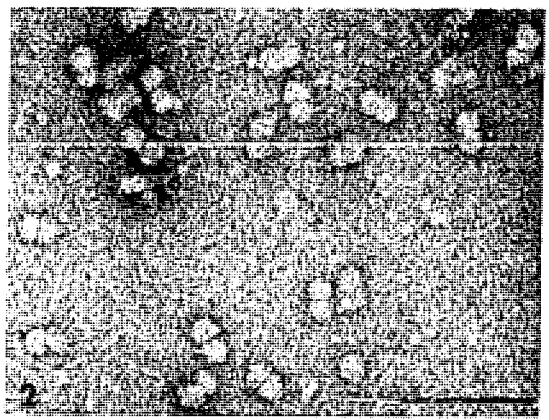

Fig. 2. Geminate particles of eggplant yellow mosaic virus observed in purified preparations stained with 2% uranyl acetate. Scale bar = 100 nm.

ate particles had an ultraviolet light absorption spectrum typical of that of nucleoprotein (A_{260}/A_{280} value of 1.3-1.4) (Fig. 3). When employed for mechanical inoculation, the purified preparations proved infective in one out if six tomato seedlings and four out of four tobacco (Xanthi nc) seedlings. Symptoms obtained were similar to those shown by tomato and tobacco plants infected with the causal agent of EYMD by whitefly transmission.

The corresponding preparations from healthy tissue treated similarly failed to show geminate particles and infectivity.

Geminate particles about 18x30 nm in size were detected in partially purified preparations of TmLCD- or TLCD-infected tomato plants. Both preparations had an ultraviolet light absorption spectrum characteristic of that of nucleoprotein (A_{260}/A_{280} value of about 1.4).

Electron microscopy. In ultrathin sections of EYMD-infected leaves of *S. melongena, D. metel* and *L. esculentum,* loose aggregates of small spherical virus-like particles were detected in the nuclei and the vacuoles of phloem and adjacent parenchyma cells. The aggregates varied

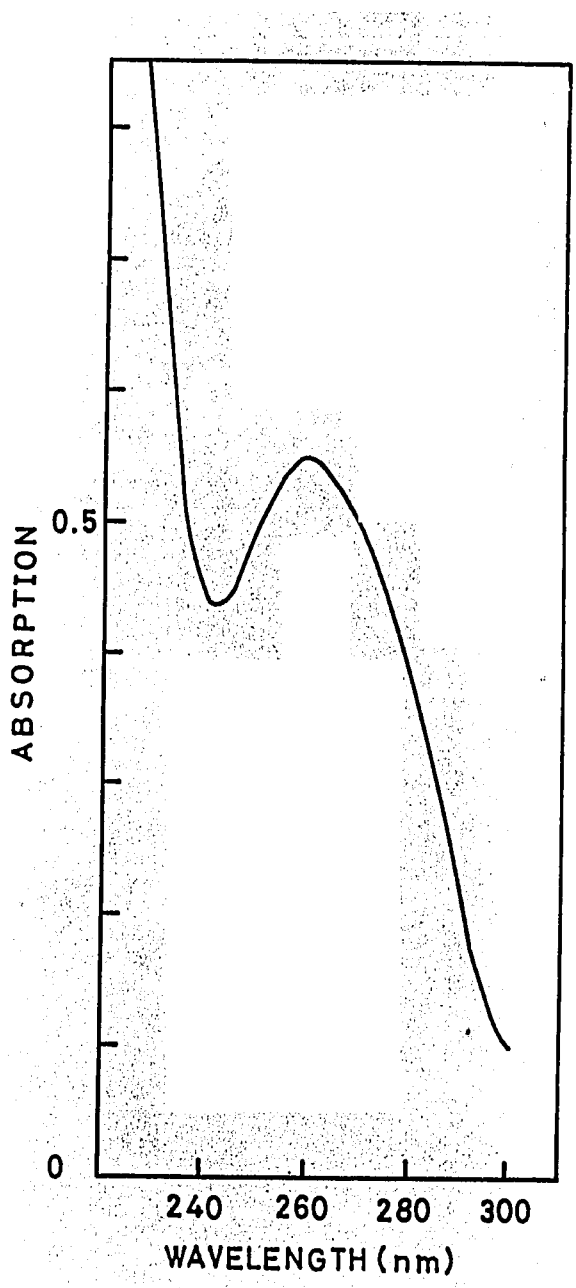

Fig. 3. Ultraviolet light absorption spectrum of purified eggplant yellow mosaic virus preparations.

Table 2. Mecahnical transmission of eggplant yellow mosaic idsease agent using different sources and test plants

Plants tested	Source plants of inoculum					Total	Infection %
	Datura metel	*Datura stramonium*	*Lycopersicon esculentum*	*Nicotiana tabacum*	*Solanum melongena*		
Datura metel	4 / 13*	2 / 11	5 / 8	–**	3 / 20	14 / 52	26.9
Datura stramonium	7 / 18	20 / 57	24 / 49	–	6 / 20	57 / 144	39.6
Lycopersicon esculentum	0 / 18	9 / 40	26 / 45	10 / 10	4 / 14	49 / 127	38.6
Nicotiana tabacum	0 / 10	1 / 15	12 / 15	10 / 10	2 / 5	25 / 55	45.5
Solanum melongena	0 / 23	0 / 19	0 / 17	–	0 / 23	0 / 82	0.0
Total	11 / 82	32 / 142	67 / 134	20 / 20	15 / 82		
Infection %	13.4	22.5	50.0	100.0	18.3		

*Number of plants infected/Number of plants inoculated; ** – : not tested.

Fig. 4. Aggregate of virus-like particles (V) occupy almost the total nuclear volume of phloem parenchyma cell of eggplant affected by eggplant yellow mosaic disease.
NM = nuclear membrane; M = mitochondrion. Scale bar = 500 nm.

in size and shape, and sometimes occupied almost the total nuclear volume (Fig. 4). These aggregates were observed in nuclei whether the nucleoli were present or not. The diameter of the individual virus-like particles was 15-20 nm. The ultrastructural changes observed were hypertrophied nucleoli of some phloem cells. No ultrastructural changes or virus-like particles were found in comparable healthy tissues.

In ultrathin sections of TmLCD- or TLCD-infected leaves of *L. esculentum*, *N. tabacum* and *P. vulgaris*, similar spherical virus-like particles were also observed in the nuclei of phloem and adjacent parenchyma cells. The virus-like particles were either scattered in the sieve tubes or as loose or paracrystalline aggregates. Hypertrophied nucleoli and fibrillar bodies with the shape of either solid circles or rings were occasionally observed in the nuclei of phloem and adjacent parenchyma cells.

DISCUSSION

Evidence from the studies on the symptomatology, host range, transmission, geminate particles found in purified preparations with infectivity and ultrathin sectioning indicates for the first time that the causal agent of EYMD is a new member of geminiviruses. We have named it eggplant yellow mosaic virus (EYMV).

The results obtained here suggest that the causal agent of TmLCD in tomato and TLCD in tabacco is a strain of tobacco leaf curl virus (TLCV), a member of geminiviruses[10]. The ultrastructural abnormalities in infected phloem cell nuclei (i.e., hypertrophied nucleoli, fibrillar bodies and aggregates of virus-like particles) were similar to those reported for several other whitefly-borne geminiviruses[3,5,6,9].

Costa[2] divided the symptoms induced in plants affected by whitefly-borne diseases into three main types; yellow mosaic, yellowing, and leaf-curl type. The symptoms of EYMV resemble those of yellow mosaic type. The symptoms of TmLCD and TLCD in Thailand are similar to those of leaf-curl type; however, the latter shows enations on the lower surface of infected leaves. TLCD was divided into two groups according to the symptoms on tobacco plants in Sri Lanka[7]. The first type, TLCD-1, shows typical leaf curl and enations on the lower surface of leaves, while the second type, TLCD-2, develops no enations but vein curvature and rugose symptoms. It seems that TLCD observed in Thailand belongs to the first type and TmLCD is related to the second type. The experimental data in the past indicated that there may be a number of strains of TLCD responsible for the development of the leaf curl disease in tomato[9,13,15,17].

Further studies are necessary to identify and classify whitefly-borne viruses among the causal agents of tobacco leaf curl, tomato leaf curl, tomato yellow leaf curl and tomato yellow mosaic diseases[1,7,8,9,11,13,16].

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TOMATO YELLOW LEAF CURL VIRUS IN THAILAND

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Our studies on tomato yellow leaf curl disease indicated that TYLCV was the cause of this most serious virus disease of tomato in Thailand. Tomato yellow leaf curl virus is different from tomato golden mosaic virus in Brazil and tomato yellow mosaic virus in Venezuela as the latter two can be mechanically transmitted[1 3]*. The rapid increase of the whitefly population and lack of a TYLCV resistant tomato variety resulted in severe outbreaks of this disease throughout Thailand.*

摘　要

番茄捲葉病爲泰國最嚴重之毒素病，研究指出是由番茄捲葉病毒(TYLCY)造成。其與巴西發生的嵌紋病毒(tomato golden mosaic virus)以及委內瑞拉的嵌紋病毒(tomato yellow mosaic virus) 不同，後二者均能經由機械傳播。粉蝨族群的快速增殖與缺少對 TYLCV 抗病的品種，爲造成泰國全國性發病的主要原因。

摘　要

タイ國のトマトに發生する最も被害の激しいウイルス病はtomato yellow leaf curl virus によるtomato yellow leaf curl病である。

ブラジルに發生するtomato golden mosaic virusおよびベネズエラに發生するtomato yellow mosaic virusは汁液傳染するので本ウイルスとは異なる。コナジラミが急速に増殖したこと及びトマトに本病に對する抵抗品種がないことによつて本病がタイ全國に蔓延することなつた。

TOMATO YELLOW LEAF CURL VIRUS IN THAILAND

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INTRODUCTION

Tomato is becoming an economically important crop in Thailand. The total tomato planting area throughout the country is over 5,000 ha. with average yield of 1,820 kg/ha. Plant diseases and pests are the major factors affecting tomato production in the tropics. Among plant diseases, tomato yellow leaf curl disease is considered the most important virus disease of tomato in Thailand, and many other countries[1].

The objectives of this paper are to report research findings and observations on tomato yellow leaf curl virus (TYLCV), the causal agent, and discuss its disease control program in Thailand.

TOMATO YELLOW LEAF CURL

Symptomatology

Infected tomato plants observed showed characteristic symptoms of leaf curling, yellowing of interveinal area, severe stunting, and flower dropping. Symptom severity depended on varieties and ages of tomato plants. Generally, table tomatoes were severely affected by the disease, especially when infections occurred prior to flowering stage. Infections of young seedlings may result in 80-100% yield losses in most of the commercially grown tomato varieties. Symptoms of tomato yellow leaf curl disease in Thailand were similar to those reported in Israel[5].

Cytopathology

Light and electron microscopic observations of infected cells revealed an excessive proliferation of phloem cells in veins, veinlets and petioles (Fig. 2A-D). Fibrillar rings as well as virus particles were found in nuclei of infected phloem cells suggesting that TYLCV was a member of geminivirus group[14].

Transmission by Vectors

The whitefly vector, *Bimisia tabaci* Genn. is commonly found in Thailand and other tropical and subtropical countries. It has a fairly wide host range of approximately 74 plant species[10]. Our study showed that *B. tabaci* can be readily established on *Nicotiana tabacum, N. glutinosa, Datura stramonium, Capsicum frutescens, Cucurbita moschata, Gossypium hirsutum, Ipomoea batatus,* and *Lycopersicon esculentum.*

The whitefly lays a cluster of eggs underneath the leaf. The eggs are slender, yellow in color, and attached to the leaf by short stalks Scanning electron microscopic observation of the first instar lavae revealed an excretory orifice and two seta at the posterior end which are characteristic of the species. The adult whitefly has two pairs of wings with 1-2 veins per wing, and a piercing-sucking mouth stylus.

Virus-vector Relationships

Tomato yellow leaf curl virus is transmitted by the whitefly in a circulative manner[4]. The acquistion period is 15-30 min., the incubation period 21-24 hr , and the transmission period at least 15 min. Symptoms develop on inoculated seedlings 2-3 wk after insect feeding. In Saudi Arabia, it has been shown that the incidence of tomato yellow leaf curl disease depended on the

whitefly population[8]. No transovarial passage of the virus was observed from viruliferous whiteflies[4].

TOMATO YELLOW LEAF CURL VIRUS (TYLCV)

Transmission

TYLCV can not be mechanically transmitted. It can however be transmitted by tissue implantation and whitefly vector[14]. No seed or soil transmission was observed[11,7].

Host Range

TYLCV can infect only solanaceous plants. *Datura stramonium* and *N. glutinasa* plants could be experimentally infected with TYLCV by tissue implantation method. Infected plants showed leaf yellowing, curling, flower dropping, and stunting[14].

Virus Purification

A modification of the purification method described by Osaki and Inouye[12] provided satisfactory results; extraction of frozen infected tissues in 0.2 m borate buffer, pH 8.5 containing 0.1% 2-mercaptoethanol and 1% antifoam A emulsion, the crude sap was expressed through a two layer cheesecloth filter and clarified with 10% n-butanol, after low speed centrifugation, the virus in supernatant was precipitated by adding 6% polyethylene glycol (MW 6000) and 1% triton X-100, followed by low speed centrifugation, the resuspended virus suspension was subjected to one cycle of differential centrifugation. This method yielded purified virus 80 ug/100 g tissue (E260 = 7.7). The $A_{260/280}$ ratio of purified virus suspension was 1.48.

Particle Morphology

Virus particles were readily degraded when stained with 2% phosphotungstic acid (PTA). Glutaraldehyde fixation and uranyl acetate staining satisfactorily preserved virus particles for electron-microscopic observations. Geminate particles, 18x30 nm in size were commonly observed in purified virus suspension. However, single particles (18 nm) and trimer particles (18x42-45 nm) were also present in virus preparations. Morphological study of TYLCV, particles strongly indicated that it was a member of geminivirus group.

Immuno-electronmicroscopy (IEM)

The IEM methods including clumping[3], Derrick[6], and decorate[9] were tested with TYLCV using the antiserum produced against the Thai isolate. Positive reactions were recorded in all tests. Derrick method proved to be the most effective and useful method for TYLCV detection.

CONTROL

Tomato yellow leaf curl disease in Thailand can be classified into two categories. First, control of the whitefly, *B. tabaci* by applications of systemic insecticides by soil drenching and regular spraying during the seedling stage. Second, cultural practices, escape cropping is used successfully in some areas. Roguing of infected seedlings is effective in reducing the spread of the disease. Tolerant tomato varieties such as KU-porter and SVRDC-4 have been planted in the areas of high disease incidence. No commercial tomato variety grown in Thailand is resistant to TYLCV.

CONCLUSION

Chemical control of the insect vector has not been effective and possibly causes an environmental hazard. A modern plant disease control scheme including the production of TYLCV resistant tomato plant by genetic engineering and the immunization of newly developed plants by using an attenuated strain of TYLCV or segment of its genome able to give cross protection should be developed.

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DISCUSSION

Q. (C.N. Roistacher)
Has this disease been reported in the same form with the geminivirus anywhere else in the world?

A. Yes. According to our information this disease is very similar to the disease spreading in the Middle East, in Isreal, Saudi Arabia, Lebanon and other countries. We have not done any serological tests as yet but from the reports I am fairly sure that they are more or less the same.

CONTROL OF CMV MOSAIC DISEASE OF TOMATO BY PRE-INOCULATION OF CMV-SR STRAIN

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SUMMARY

A virus isolated from spinach plants showing rugose symptoms was identified as cucumber mosaic virus (CMV), and named CMV-SR. The virus did not show any symptoms on systemically infected tomato plants, therefore, the virus seemed to be useful as attenuated CMV for controlling CMV diseases on tomato plants. In greenhouse tests, CMV-SR effectively protected against infection of virulent strain on tomato plants in sap inoculation and also aphid transmission of virulent strain. In field tests, the pre-inoculation of CMV-SR reduced the percrentage of mosaic and spinal leaf diseases on tomato plants.

摘　要

由菠菜分離出，並產生皺葉病徵之一種病毒，已被證實爲胡瓜嵌紋病毒(CMV)，本文中以CMV - SR稱之。此病毒在感染之番茄植株並不顯現病徵，因此似乎可視爲毒力減弱的CMV，而用於防治番茄上發生的胡瓜嵌紋病。在温室利用汁液接種或蚜蟲傳播之測驗中，CMV-SR能有效防治強烈型CMV番茄胡瓜菠紋病。田間試驗指出經CMV-SR接種後，可以降低番茄嵌紋與捲葉之百分比。

摘　要

退葉症狀を示すホウレンソウから單離されたウイルスはCMVと同定されCMV-SR と命名された。本ウイルスはトマトに全身感染するが無病徴である。その爲弱毒ウイルスとしてトマトのCMVによるモザイク病の防除に使える可能性が考えられた。温室實驗ではCMV-SRは強毒ウイルスの汁液接種またはアブラムシ傳搬によるトマトの感染を效果的に防止した。圃場試驗ではCMV-SRの前接種によりトマトのモザイク、針狀葉株率を減少させた。

CONTROL OF CMV MOSAIC DISEASE OF TOMATO BY PRE-INOCULATION OF CMV-SR STRAIN

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INTRODUCTION

Tomato plants are grown in fields in spring and autumn in Japan. Cucumber mosaic virus (CMV) diseases causing mosaic, spinal leaf and necrotic streak are one of the most important diseases of tomato plants.

Farmers spray insecticides to control aphids that are vectors of CMV; however, the control is ineffective because aphid vectors immigrate into tomato fields adjacent areas, and also the transmission mode of CMV by aphids is of a non-persistent type.

For these reasons, researchers have looked for an attenuated strain. This paper describes a mild strain of CMV found for use as an attenuated virus in the control of CMV disease of tomato plants.

MATERIALS METHODS AND RESULTS

A virus isolated from spinach plants showing rugose symptom in Saitama Prefecture, Japan, was identified as cucumber mosaic virus (CMV) based on host range, aphid transmissibility, particle morphology, and serological relationships. The virus was serologically indistinguishable from CMV-P strain[1]; however, electrophoresis of RNA of the virus revealed four components showing minor differences in mobility from the components of CMV-Y and CMV-P. From these results we proposed the name of CMV-SR for the virus[2].

The virus infected 20 plant species of seven families, among 38 species of 13 families tested by sap inoculation. The susceptible plant species, *Spinacia oleracea*, *Beta vulgaris*, *B. vulgaris* var. *flacescens*, *Nicotiana clevelandii*, *N. debneyi*, *N. glutinosa*, and *N. tabacum* cv. Samsun showed mild systemic symptoms, including mild mosaic and rugose, however, these symptoms were milder than those caused by other CMV strains or isolates reported in Japan. And also, the virus infected systemically without symptoms on *Lycopersicon esculentum* (tomato), *N. tabacum* cvs. Bright Yellow and Xanthi nc. *Cucumis sativus*, *Gomphrena globosa*, *Chrysanthemum coronarium*, *Zinnia elegans* and *Petunia hybrida*. *Chenopodium amaranticolor*, *C. quinoa*, *Vigna sesquipedalis* cv. Kurodane Sanjaku, and *V. unguiculata* cv. Black Eye showed small local lesions in inoculated leaves. *Tetragonia expansa* was infected with the virus in inoculated leaves without showing symptom. The other 18 plant species of 9 families were not infected with the virus (Table 1).

The results of this host range study suggested that the virus might be a useful attenuated virus for controlling the CMV diseases of tomato plants.

CROSS PROTECTION OF CMV-SR TO VIRULENT STRAIN

Tomato cultivars used in these experiments were known to be resistant to tobacco mosaic virus (TMV); this was to avoid TMV contamination. Inoculation of virulent CMV in crude juice of *N. tabacum* cv. caused bright yellow leaves about seven days after inoculation.

Table 1. Host range of CMV-SR

Plant	Symptoms inoc.	non-inoc.
Beta vulgaris	1	LC
B. vulgaris var. *flacescens*	1	LC
Chenopodium amaranticolor	L	–
C. qunioa	L	–
Spinacia oleracea	1	R
Gomphrena globosa	1	s
Tetragonia expansa	1	–
Brassica oleracea	–	–
B. pekinensis	–	–
B. rapa	–	–
Raphanus sativus	–	–
Glycine max	–	–
Phaseolus vulgaris	–	–
Vicia faba	–	–
Vigna sesquipedalis	L	–
V. unguiculata	L	–
Capsicum annuum	1	M
Lycopersicon esculentum	1	s
Nicotiana clevelandii	1	s
N. debneyi	1	m
N. glutinosa	1	m
N. tabacum cv. Bright Yellow	1	s
N. tabacum cv. *Xanchi* NC	1	s
N. tabacum cv. Samsun	1	m
Petunia hybrida	L	s
Solanum melongena	–	–
Hibiscus esculentus	–	–
Sesamum indiucm	–	–
Citrullus vulgaris	–	–
Cucumis melo	–	–
C. sativus	1	s
Cucurbita moschata	–	–
Chrysanthemum coronarium	1	s
Lactuca sativa	1	s
Zinnia elegans	1	s
Daucus carota	–	–
Allium fistulosum	–	–
Lolium multiflorum	–	–
Sorghum vulgare	–	–
Zea mays	–	–

L: local lesion
R: rugose
M: mosaic
–: no infection
1: symptomless infection on inoculated leaves

LC: leaf curling
m: mild mosaic
s: symptomless infection on non-inoculated leaves

Experiment I.

Inoculum of CMV-SR was prepared by grinding *N. debneyi* leaves showing mosaic symptoms with five volumes of 0.05 M phosphate buffer, pH 7.0, containing 0.02% KCN. This inoculum was tested by inoculation to leaves of *C. umaranticolor* on which it produced more than 2000 local lesions.

The CMV-SR was inoculated to cotyledons of two week old tomato seedlings and did not cause any mosaic or spinal leaf symptoms on these tomato plants. However, inoculation by CMV virulent strain to two week old seedlings did cause these symptoms.

In two further treatments of the CMV-SR inoculated seedlings, two groups of twelve plants were inoculated with virulent strain at 10 and 26 days after the initial CMV-SR inoculation. In the 10 day post-inoculation treatment no plants developed mosaic or spinal leaf symptoms in result of virulent strain inoculation, and, in the 26 day post-inoculation treatment only two plants developed the symptoms. This result revealed that the pre-inoculation with CMV-SR gave strong cross protection against infection by the virulent CMV.

Experiment II.

In three treatment groups of two week old tomato seedlings, their cotyledons were inoculated with inocula of purified CMV-SR made up to 3, 6 or 30 ug per ml, respectively. A control group was maintained (Group IV).

In a sap inoculation test, two sets of twelve plants from each of the first three groups were inoculated with virulent strain 14 or 23 days after CMV-SR pre-inoculation. Two sets from the controls (Group IV) were similarly sap inoculated, six plants at 14 days and three plants at 23 days, respectively. A further set of six plants from each of the first three treatments were maintained as controls.

These latter three sets showed no symptoms, being inoculated with CMV-SR only. Of those sets sap inoculated with CMV virulent strain at 14

days, in the CMV-SR 6 and 3 ug pre-inoculated plants one plant in twelve showed mosaic and spinal leaf symptoms; however, in the 30 ug pre-inoculated set three plants expressed symptoms, whereas in the control group all plants showed symptoms. Of those sets sap inoculated with CMV virulent strain at 23 days, in the CMV-SR 30 and 3 ug pre-inoculated plants one plant in each set of twelve showed symptoms; however, in the 6 ug pre-inoculated plants three of twelve showed symptoms, whereas again all plants in the control set showed symptoms (Table 2).

Table 2. Cross protection of CMV-SR to sap-inoculated virulent strain (Experiment II)

Group	Virus	Infection Rate		
Date	April 20th	May 4th	May 13th	June 21st
I-1	CMV-SR 30 μg	–	–	0/6
I-2	"	CMV	–	3/12
I-3	"	–	CMV	1/12
II-1	CMV-SR 6 μg	–	–	0/6
II-2	"	CMV	–	1/12
II-3	"	–	CMV	3/12
III-1	CMV-SR 3 μg	–	–	0/6
III-2	"	CMV	–	1/12
III-3	"	–	CMV	1/12
IV-1	–	CMV	–	6/6
IV-2	–	–	CMV	3/3

$$\text{Infection Rate} = \frac{\text{No. of diseased plants}}{\text{No. of tested plants}}$$

Aphid transmission tests were carried out by using *Myzus persicae* reared on turnip plants (Table 3). Aphids after fasting for two hours in glass beaker, were transferred to diseased tobacco plants for acquisition access of 15 min. Then, 10 aphids per plant were transferred to sets of six tomato plants taken from the pre-inoculated and control groups fourteen days after pre-inoculation, for inoculation access of one day after which the aphids were removed by spraying insecticide. Of six tomato plants with no pre-inoculation, inoculated with virulent strain by aphid transmission, two showed mosaic and spinal leaf symptoms. On the other hand, tomato plants inoculated with virulent strain by aphid transmission at 14 days after the pre-inoculation of CMV-SR (3, 6 or 30 μg per ml) showed mosaic and spinal leaf symptoms on 0, 1 and 1 plants among each 12 plant group, respectively. Again pre-inoculated controls showed no symptoms.

Table 3. Cross protection of CMV-SR to aphid-transmitted virulent strain

Group	Virus	Infection Rate	
Date	April 20th	May 4th	June 21st
I-1	CMV-SR 30 μg	–	0/6
I-2	"	CMV	1/12
II-1	CMV-SR 6 μg	–	0/6
II-2	"	CMV	1/12
III-1	CMV-SR 3 μg	–	0/6
III-2	"	CMV	0/12
IV	–	CMV	2/6

Ten aphids per plant

$$\text{Infection rate} = \frac{\text{No. of diseased plants}}{\text{No. of tested plants}}$$

These results showed that the pre-inoculation of CMV-SR protected against infection of virulent strain on tomato plants even if the concentration of CMV-SR in inoculum was as low as 3 μg per ml; however, protection was not complete. Therefore, the inoculum containing 100 μg of purified CMV-SR per ml was tested.

Experiment III.

Inoculum containing 100 μg of purified

CMV-SR per ml was inoculated on cotyledons of two week old tomato seedlings and these tomato seedlings were inoculated in different groups of six with virulent strain by sap or aphid transmission at 5, 10 or 21 days after pre-inoculation of CMV-SR. Six other plants were inoculated with virulent strain only.

Tomato plants pre-inoculated as seedlings with CMV-SR did not show any symptoms. In sap inoculation tests of virulent strain, the six tomato plants inoculated with virulent strain all showed mosaic and spinal leaf symptoms. On the other hand of six tomato plants in each group inoculated with virulent strain at 5, 10 or 21 days after pre-inoculation of CMV-SR 0, 0 and 5 plants, respectively showed mosaic and spinal leaf symptoms. In the last case, the concentration of virulent strain in inoculum might have been too high. In a parallel test using aphid transmission of virulent strain, tomato plants with out pre-inoculation inoculated with virulent strain by aphids showed mosaic and spinal leaf symptoms on 4, 3 and 3 plants, respectively. On the other hand, tomato plants inoculated with virulent strain by aphids on 5, 10 or 21 days after pre-inoculation of CMV-SR did not show any symptoms.

FIELD TRIALS OF CMV-SR PRE-INOCULATION

Glasshouse tests suggested that CMV-SR was useful as attenuated virus for controlling CMV diseases in tomato plants. Inoculum containing 100 μg of purified CMV-SR per ml was used in field tests and inoculated to cotyledons and first foliage leaves of tomato seedlings. Field tests were carried out in spring to summer and summer to autumn.

(1) The concentration of CMV-SR in tomato plants.

Tomato plants at first foliage leaf stage were inoculated with CMV-SR (100 μg per ml) on April 6 and August 9. The inocula produced 178 and 137.7 local lesions per cm^2 on inoculated leaves of *C. amaranticolor*, respectively. The concentration of CMV-SR in the upper leaves of tomato plants inoculated with CMV-SR was analyzed by number of local lesions on inoculated leaves of *C. amaranticolor*. The leaves were ground with 10 volume of 0.05 M phosphate buffer, pH 7.0, containing 0.02% KCN. Juice from upper leaves of these tomato plants produced less than five local lesions per cm^2 on inoculated leaves of *C. amaranticolor* up to about 80 days after the inoculation of CMV-SR, showing the concentration of CMV-SR in tomato plants to be low.

(2) Control of CMV diseases on tomato plants in field.

Tomato plants inoculated with CMV-SR were transplanted to the field, and observed for symptoms for about 80 days after transplanting.

In the spring to summer experiment tomato plants, transplanted in the field on May 15, showed mosaic and spinal leaf symptoms on 58% of the plants about one month later, and the percentage of plants showing mosaic and spinal leaf symptoms reached 75% in middle of July.

Although, tomato plants pre-inoculated with CMV-SR (100 μg per ml) showed mosaic and spinal leaf symptoms about one month after transplanting in field; however, the precentage of diseased plants was very low about 7% at one month, and 19% by the middle of July. Moreover, the number of fruit showing abnormal colour was only half of that of tomato plants in the controls. The growth of tomato plants inoculated with CMV-SR was slightly reduced in comparison with that in controls at the early stage; however, after one month growth was almost same as in controls.

In the summer to autumn experiment tomato plants transplanted in the field on September 7, showed mosaic and spinal leaf symptoms in 16% after one month, and the disease incidence increased to 48% by end of November.

Tomato plants pre-inoculated with CMV-SR also showed mosaic and spinal leaf symptoms one month after transplanting, the percentage of

diseased plants was lower than that in controls and the percentage of diseased plants at end of November was only 12%, about one fourth of that in controls. The growth of tomato plants inoculated with CMV-SR was reduced slightly in comparison with that in controls; however, after one month was almost same.

DISCUSSION

As described above, the pre-inoculation of CMV-SR on tomato seedlings reduced the occurrence of CMV diseases causing mosaic and spinal leaf symptoms, and also tomato plants inoculated with CMV-SR produced normal colored and a higher quantity of fruit than controls of which 48-75% were infected with CMV showing mosaic and spinal leaf symptoms.

Some of tomato plants inoculated with CMV-SR showed mosaic and spinal leaf symptoms. In these plants CMV-SR infection may not have taken, because back inoculation to *C. amaranticolor* showed that these plants were only infected with virulent strain. Therefore, inoculation technique of CMV-SR must be improved.

CMV-SR is not able to be applied in the field around spinach or pimiento crops because CMV-SR is transmissible by aphids and causes rugose and mosaic symtpoms on these plants. More detailed host range studies should be conducted before the application of CMV-SR pre-inoculation in farmers' fields. A non aphid-transmissible attenuated CMV, would solve this problem.

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DISCUSSION

Q. (H.J. Chiu)
Your data seems to indicate that the protective strain offers the tomato better protection against the severe strain in summer than in winter. Is the period of protection longer in the summer than in winter?

A. In the summer the farmers cultivate in the open field where as in winter they cultivate in plastic greenhouses, and so in winter there is no serious infection problem.

Q. (H.J. Chiu)
But is your data then from these greenhouses?

A. No. The data indicated 'field', is all open field. Crop protection only was tested in the greenhouse.

Q. (Simon W.Y. Wang)
After inoculation of CMV-SR strain and then CMV severe strain challenging inoculation, could you isolate both strains from the inoculated plants; or could you only isolate the CMV-SR strain?

A. I inoculated the CMV-SR strain, and then later challenged with the virulant strain, and after some days I checked the tomato plants for inoculate, in which case we can detect only SR strain or severe strain, we cannot detect both strains.

Q. The reason that I asked the question is that I am interested in the mechanism of cross protection. In this case if we cannot isolate both strains from the inoculated plant, is that because the protection was to suppress the second strain in replication or were both viruses replicated in the plant but only the symptom suppressed?

A. I cannot answer that. I do not know the actual mechanism, but we think that the challenge virus is supressed in virus replication in the CMV-SR inoculated tomatoes.

Q. (H.J. Chiu)
Does the phenoma of cross protection have anything to do with the satellite RNA of CMV? Is it possible that the protecting strain has a satellite RNA, which the severe strain does not have?

A. This virus strain does not have a satellite virus. SR strain has only four RNAs, not five.

Q. (S.D. Yeh)
You mentioned that the SR strain has only four RNAs. After you inoculated the virus to tomato did you check again whether an additional RNA 5 could be found?

A. I do not think there is an RNA 5.

Q. (S.D. Yeh)
I ask because we know that there is one strain found at New York State Agricultural Experiment Station, a bean strain of CMV, which also causes symptomless infection in tomato, and this strain does contain RNA 5. RNA 5 there suppresses expression of the severe symptom. So I am not sure whether the result is due to a cross protection between your protective strain of virus and the challenge strain or is due to the interference of a similar RNA 5. Sometimes with these CMV associated RNA 5 you cannot detect them in certain hosts. They have to be passed through potato, tomato or certain cucumber squash, in order to be detected. Do you think your protection is due to the cross protection mechanism or do you think may be there is some effect of RNA 5?

A. I expect that there was no change in the virus RNA content.

VIRUS DISEASES OF TOMATO AND CHINESE CABBAGE IN TAIWAN AND SOURCES OF RESISTANCE

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SUMMARY

The most important viruses of tomato in Taiwan are tomato mosaic virus (ToMV strains 0 and 1), potato virus Y (PVY) and cucumber mosaic virus (CMV). Potato virus (PVX), tobacco etch virus (TEV) and a gemini-type virus causing yellowing and leaf-curling have been isolated occasionally. Another, possibly a new potyvirus, is also present. Turnip mosaic virus (TuMV) and cucumber mosaic virus (CMV) are the only viruses, reported from Chinese cabbage. The four known strains of TuMV and a new strain, C-5, have been isolated from Chinese cabbage and other Brassica crops. At AVRDC, breeding for resistance to ToMV is underway, as well as a search for sources of resistance tc PVY and CMV. One wild type tomato, L. hirsutum has already been found with immunity to PVY. AVRDC's Brassica sp. germplasm collection is presently screened for resistance to TuMV and sources of resistance to one or several strains have already been identified in some Chinese cabbage lines. The resistance mechanism is under investigation.

摘　要

在臺灣，感染番茄最重要的病毒，爲番茄嵌紋病毒(ToMV, strains 0 and 1)，馬鈴薯病毒Y(PVY)及胡瓜嵌紋病毒(CMV)。除此以外，偶然分離得到的病毒有馬鈴薯病毒X(PVX)，菸草蝕刻病毒(TEV)以及一種會造成葉片黃化與捲葉病徵的Gemini-type 病毒，其病毒顆粒通常成對出現。另外，還分離出一種可能屬於新的PVY群病毒。

在結球白菜上已被發現的病毒僅有蕪菁嵌紋毒素(TuMV)及胡瓜嵌紋病毒兩種。前者包括四個已知的病毒系統，以及第五個新的系統，係從結球白菜及其他Brassica屬蔬菜上分離得到。

亞蔬中心已開始進行番茄嵌紋病毒(ToMV)的抗病育種工作，同時積極尋找(PVY)及(CMV)的抗病種源。一種野生番茄*L. hirsutum*已被發現有對(PVY)的抗性。對蕪菁嵌紋病毒某一系統或多個系統的抗病材料，目前已從亞蔬中心十字花科種源中篩選出來。其抗病機制正在探討中。

摘　要

台湾におけるトマトの重要ウイルス病はTomato mosaic virus (ToMV strains 0 and 1) potato virus Y (PVY) cucumber mosaic virus (CMV) である。potato virus X X (PVX) tobacco etch virus (TEV) 黄化、リーフカールをおこすジエミニウイルスが時々分離される。この他に新しいpotyvirus と思われるものがある。白菜から分離されるのはturnip mosaic virus (TuMV) とcucumber mosaic virus だけである。TuMV の既知の4系統と新系統、C-5が白菜やその他のアブラナ科植物から分離された。AVRDCではToMVに對する抵抗性育種が進行中であり、PVY, CMV に對する抵抗性遺傳子源も探されている。野性トマトの1種、*L. hirsutum* が PVY に免疫であることが發現された。AVRDC のアブラナ科植物遺傳子源收集ではTuMVに對する抵抗性と既に或種の白菜の系統發見されている1～數種のウイルス系統に對する抵抗性の遺傳子源に對する檢定を實施中である。抵抗性の機構を研究中である。

VIRUS DISEASES OF TOMATO AND CHINESE CABBAGE IN TAIWAN AND SOURCES OF RESISTANCE

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INTRODUCTION

Tomato and Chinese cabbage are two important vegetable crops in Taiwan. Of the total 232,655 hectares of land under vegetable production, 15,545 hectares are planted to Chinese cabbage which includes both the heading and the non-heading types. This is the second largest area planted to a single vegetable crop, second only to that of bamboo shoots, which comprises the biggest production area of 24,118 hectares. The area grown to tomato (including fresh market and processing type tomatoes) with 12,394 hectares ranks third[2].

Both tomato and Chinese cabbage are affected by a number of diseases many of which are effectively controlled in Taiwan by chemicals and cultural practices[47]. Virus diseases on the other hand are still abundant because of the continuous presence of the viruses, their vectors and host plants. This is in part due to the intensive and continuous multiple cropping farming system, which has been traditionally practiced in Taiwan, and because of the lack of varieties with resistance to the particular viruses (and strains) which are endemic under Taiwan's agroclimatic conditions[47].

VIRUSES OF TOMATO

A number of viruses have been reported on tomato in Taiwan, the most important and widely distributed ones being TMV, PVY and CMV. Of the other viruses known to affect tomato, only potato virus X (PVX), tobacco etch virus (TEV) and a leafcurl virus have been found occasionally. Another, possibly a new virus of the potyvirus group is also present.

Tomato Mosaic Virus (ToMV)

ToMV is known to occur as a number of strains. In the USA and Europe, five major strains, ToMV-0, ToMV-1, ToMV-2, ToMV-1.2 and ToMV-2[2] have been recognized, the most common being ToMV-0 and ToMV-1[6,12,17]. Little information is available on the strain situation in Southeast Asia.

In Taiwan, tomato mosaic virus was first reported in 1944[1]. Later, in 1975 intensive electronmicroscopic investigations of ToMV infected tomato were conducted[19] and unusual angled layer-aggregates were found, which differed from those previously reported for TMV. It was suggested then that the isolate used in this study was a new strain of TMV, closely related to the aucuba strain.

Tomato mosaic virus (ToMV) is quite prevalent on tomato in Taiwan. It was found in 50% of the leaf samples with virus-like symptoms collected during 1980-1982[3] from fresh market and processing tomatoes in 10 out of 15 counties surveyed (Table 1). Strain classification, based on Rast's three *L. esculentum* differentials,[49] CSTMW-18, Delissa and Perou 2 (Table 2) indicated that ToMV-1 and ToMV-0 are the two most common strains, occurring in almost equal frequency. Of the 223 ToMV isolates strain-typed, 50% were ToMV-0 and 49% were ToMV-1. ToMV-2 was found to be present also, but in very low frequency. It was accidentally isolated from three samples originating from Tainan county, which

Table 1. Occurrence of ToMV in the tomato production areas of Taiwan

Counties surveyed	10
Counties where ToMV was detected	10
Total no. of samples collected (fresh market and processing type tomatoes)	587
Samples containing ToMV	223/587 (38%)
Samples containing ToMV strain 0	112/223 (50%)
Samples containing ToMV strain 1	108/223 (48%)
Samples containing ToMV strain 2	3/223 (1.4%)
Samples containing ToMV strain 2^2	0/223 (0%)
Samples containing other ToMV strains	0/223 (0%)

were infected with leafcurl virus[3]. To obtain the leaf curl virus without the contaminating ToMV, the tomato cultivars Delissa (genotype $Tm2^2/Tm\ 2^2$) and Moperou (genotype Tm2/Tm2), both resistant to strains 0 and 1 (at that time the only ToMV strains detected in Taiwan) were grafted onto the leafcurl infected tomato plants for the elimination of contaminant ToMV. However, the Moperou scions developed mosaic in addition to leaf curl symptoms and ToMV-2 was subsequently isolated from these plants[3,4]. ToMV-2^2 and the other known strains of ToMV have not been isolated. Both ToMV-1 and ToMV-0 were also found in sweet pepper and chili pepper samples. ToMV-0 was also found in *Solanum nigrum*[3].

Table 2. The reactions of Rast's *L. esculentum* differentials to eight strains of ToMV

Differential cultivars (genotype)	ToMV 0	1	2	2^2	1.2	1.2^2	2.2^2	$1.2.2^2$
GCR (tm/tm)	+	+	+	+	+	+	+	+
CSTMW-18 (Tm-1/Tm-1)	–	+	–	–	+	+	–	+
Delissa ($Tm\text{-}2^2/Tm\text{-}2^2$)	–	–	–	+	–	+	+	+
Perou 2 (Tm-2/Tm-2)	–	–	+	–	+	–	+	+

Virus infected seed and plant debris in the soil are generally considered the most important sources of ToMV[6]. This is true also for Taiwan[3], where 19 of 23 seedlots of tomato cultivars, originating from the major commercial seed companies and AVRDC, were found to be contaminated with ToMV strains 1 and 0 (Table 3). The same strains were also recovered in 18 soil samples taken up to a depth of 20 cm from 85 fields at various locations in Tainan county, one of Taiwan's major production areas of processing tomatoes[3]. The surveyed fields either had tomatoes growing at the time of sampling or had been planted to tomato at various times prior to sampling. Interestingly, ToMV was detected in the soil only up to 5 months after the tomato harvest. In soil samples of fields where tomato had been harvested 6-16 months prior to sampling,

ToMV was not found (Table 4). In countries of the temperate zone, survival of ToMV in the soil is reported to be much longer, up to several years[6,7]. The reasons for the comparatively short survival of ToMV in the soils under southern Taiwan conditions are not clear. The type of soil is known to affect survival of ToMV in the soil[25]. Higher soil and atmospheric temperatures and different crop rotation patterns which often include flooding of the soil during a paddy crop may be responsible for this short survival of ToMV in the soil. In preliminary studies, however, time of survival of ToMV did not differ much between non-flooded soil and artificially flooded soil amended with ToMV infected plant debris[3]. ToMV was detected in the flooded soil up to 6 months after the amendment in January, and in the non-flooded soil up to 5 months. Large amounts of ToMV were found up to 3 months in both flooded and unflooded soils. A sharp decline in ToMV presence in both soils occurred after the fourth month which coincided with the onset of warmer air temperatures. After 7 months, no ToMV was detectable in either soil.

Table 3. Occurrence of ToMV on seed of commercial cultivars and AVRDC breeding lines

Seed[1] source	Cultivar	No. of local lesions produced on half-leaves of *N. tabacum* 'Xanthi'[2]	ToMV strain
A	1	50	0
	2	50	0
	3	4	1
	4	50	0
	5	50	1
	6	11	1
	7	1	1
	8	50	0
B	1	2	1
	2	1	1
	3	0	–
	4	0	–
	5	2	0
C	1	21	0
	2	46	0
	3	27	1
D	1	1	0
	2	1	0
	3	2	0
E	1	0	–
	2	1	1
	3	0	–
	4	4	0

[1] Commercial seed companies of Taiwan and AVRDC
[2] Average no. of local lesions produced from four samples of 100 seeds, each inoculated on two half leaves of *N. tabacum* 'Xanthi'

Table 4. Survival of ToMV in agricultural soils.

Sampling[1] time	No. of fields with ToMV/ No. of fields sampled
0[2]	7/8
1	2/3
2	1/4
3	4/7
4	3/11
5	1/9
6	0/4
7	0/3
9	0/1
10	0/3
11	0/2
12	0/26
13	0/1
14	0/1
16	0/2

[1] Months after the tomato harvest.
[2] At cropping time.

Considering the practical impossibility of ToMV control in tomatoes by preventive measures, it is logical that breeding for resistance is the best solution of the problem.

Three single dominant genes for resistance, Tm-1, Tm-2 and Tm-2^2, originally from the green fruited species of tomato *L. peruvianum*, and *L. hirsutum*, but also from *L glandulosum*, and *L. pennellii*[41], have already been transferred to *L. esculentum* and are available in many commercial cultivars grown in temperate areas.

The Tm-1 gene offers resistance to three of the five major strains: 0, 2 and 2^2. It does, however, permit limited multiplication of the common strain[18,42]. The gene Tm-2^2 and its allele Tm-2 on the other hand, give a high level of resistance to the two most commonly occurring strains 0 and 1[51].

The mode of action of the Tm-2^2 gene and its allele Tm-2 is not clearly understood, but it is thought to either limit ToMV multiplication within cells or prevent cell to cell movement[51].

There are differences of opinion regarding the breeding policy to be followed. The Tm-2 gene alone is rarely used by the breeders because it reduces fertility[30] and favours selection of strain TMV-2 which does occur naturally at very low frequencies in nature.

On the other hand, because strain Tm-2^2 almost never occurs in nature, the gene Tm-2^2 is preferred by breeders since it is not likely that it will favour selection of TMV-2^2[,30]. Many commercially available F_1 hybrids, notably the Dutch ones, now carry the Tm-2^2 gene for resistance in the homozygous state. However the use of the highly effective Tm-2^2 resistance bears two disadvantages:

1) The Tm-2^2 gene is associated with reduced fertility of the plant[30].

2) A necrotic reaction occurs at elevated temperatures in heterozygous plants and to a lesser extent in homozygous genotypes in response to infection by common strains of the virus[10,11,12,30,43,45,49].

The necrotic symptoms will not appear however when an additional Tm-1 gene is present[30]. For this reason many French F_1 hybrids carry both resistance factors, Tm-2^2 and Tm-1 in the homozygous and heterozygous state respectively[30].

Several efficient mass-inoculation methods have been described[49]. At AVRDC, we have adopted and modified the air pressure method for mass screening. We use a 1:50 dilution of infected plant tissue in phosphate buffer (0.01 M, pH 7.0, 2% Celite added) and apply it to young seedlings at the first leaf stage at a rate of 0.5 ml per plant, using air pressure of 3.5 kg/m^3 at 10 cm distance.

Among the great number of Tm-2^2 resistant material available, we have selected a number of cultivated varieties, e.g., Ohio MR-13 as the donor of the Tm-2^2 gene for our breeding program, mainly because of their good agronomic characters. Most of AVRDC's promising heat tolerant tomato lines already carry this gene in the homozygous state. It has been introduced through conventional breeding methods, such as pedigree, SSD, bulk and more recently, by backcross. The incorporation of the Tm-1 gene into these lines has also begun recently. However selection in segregating populations of genotypes carrying the Tm-2^2 and Tm-1 genes together is difficult using inoculation with the presently available strains TMV-0, 1 or 2.

Effective selection of the above genotypes is only possible by inoculating the segregating plants with strain TMV-0 ($N2^2$)[28]. This strain is a TMV-0 strain which is necrotic on plants carrying the genotype Tm-2^2 in the homozygous condition. It will not attack plants which carry both the Tm-Tm-1 and the Tm-2^2 gene in any combination.

However this pathotype of ToMV strain 0 has not yet been found to occur in Taiwan and it is not certain whether quarantine regulations will permit introduction of this strain for use in the AVRDC screening program.

Cucumber Mosaic Virus (CMV)

CMV has frequently been found in surveys of tomato plantings in Taiwan[3,4]. Of a total of 913 leaf samples collected from 1983-1985, 33%

were found to contain CMV[4]. The symptoms associated with this virus were mottle, mosaic, and various kinds of leaf deformation, often so extreme that the leaves consisted of little more than a central rib. Often, infected plants were stunted and had small leaves. Infection could take place at any time during the growth period even when plants had already reached maturity and were bearing fruit. This was evidenced by the tip of the plant, or one of the branches. suddenly developing clearly recognizable symptoms. Farmers will usually rogue out young infected plants and replace them with healthy ones. However plants in which CMV becomes apparent at a later stage, particularly at the fruit bearing stage, are often kept in the field and thus constitute continuous sources of inoculum. Fields with more than 50% infection have been found. Infection as high as 100% was recorded in one isolated planting, surrounded on all sides by densely planted bananas.

This virus is generally not considered a major problem of tomato in the temperate zone, however, in the tropics and subtropics, where the vectors, weed hosts and cultivated hosts are present year round, continuous sources of inoculum for this virus are almost guaranteed. We have collected more than 30 isolates from tomato and have attempted to strain-type them according to Marrou's system[36]. However symptom development on the differential hosts was inconsistent and erratic under our greenhouse conditions and we were only able to roughly group our isolates[3]. One of the isolates clearly can be classified as the legume strain because it systemically infected both *Vigna* sp. and *Phaseolus* sp.

Genes for resistance have so far not been located in tomato and its wild relatives. Tolerance was found in *Solanum lycopersicoides* and *L. peruvianum* which are both symptomless carriers of the virus[28,43]. A search for possible sources of resistance in the AVRDC germplasm collection, was initiated in 1984. Mainly wild type *Lycopersicon* sp. such as *L. pimpinellifolium, L. peruvianum, L. hirsutum,* and *L. glabratum* were screened. The plants were subjected to two subsequent artificial inoculations with an isolate that produces severe shoestring symptoms on tomato (CMV-PEET). All the tested lines were susceptible[4]. However, among these was one line of *L. Peruvianum* which upon inoculation with the virus had only 50% of plants developing symptoms of infection. This line will be rescreened with a different CMV isolate. While the search for resistance in AVRDC germplasm is ongoing, six hairy tomato lines are being evaluated as a possible source of non preference or deterrence to aphids, the vector of CMV. In a laboratory study we had found that the total probe time and the total probe numbers but not the individual probe time were significantly reduced on the hairy tomato lines[3] (Table 5). This led us to assume that CMV development in these plants might be slowed down in the field. In the spring of 1984, these hairy tomato lines were planted in the field and exposed to conditions of natural infection. By the end of the growing season, the disease incidence had reached almost 100% in the nonhairy check, whereas for the hairy lines it ranged only from 33.8% to 76.3%. The infection rates (r) (the rate of disease increase per unit time *sensu* van der Plank) of the hairy tomato lines were significantly lower than that of the nonhairy line (Table 6)[4]. However, in a second field test in the spring of 1985 using the four best performing hairy lines and three nonhairy controls, these findings could not be repeated. At the end of the growing season, disease incidence of the 4 hairy lines ranged from 81% to 91% which was not significantly lower than that of the nonhairy ones, which ranged from 89 to 100%. There was also no significant difference observed in the rate of disease increase of the hairy versus nonhairy tomato lines. No correlation was found betweeen hair numbers of the upper or lower leaf surfaces at different growth stage and the disease development. The failure of the hairy tomato lines in the second experiment to support a lower rate of disease development which was evidenced in the first field trial is thought to be due to a higher disease and vector pressure in the field.

Table 5. Hair density of hairy and non-hairy tomato and its relation to the feeding behaviour of aphids

Leaf position	Hair no. (per 44.18 mm²)	Total probe time (Min)	probe no.	Average probe duration (Min) z)
Leaf 1				
Hairy	523	1.85	0.97	1.97
Non-hairy	56	4.16	2.04	1.90
Leaf 4				
Hairy	545	1.03	1.61	1.60
Non-hairy	89	2.60	3.74	1.50
Leaf 6				
Hairy	378	0.47	1.03	1.53
Non-hairy	32	1.67	2.53	1.50
Factor leaves	Factor value of difference between hairy and non-hairy and significance for each parameter			y)
Leaf 1	12.91***	3.92***	3.99***	0.41 NS
Leaf 4	12.60***	3.61***	5.82***	0.22 NS
Leaf 6	9.54***	2.53*	4.46***	0.03 NS
df.	59	179	179	17

z) Values are weighted average for each of the 3 groups of 10 apids.
y) NS = $P > 0.10$, * = $P < 0.05$, *** = $P < 0.001$.

Table 6. Reactions of Taiwan tomato PVY isolates on three hosts used to distinguish potato strains of PVY.

Differential host	Taiwan Tomato Isolates			Common Potato Strains		
	Group A	Group B	Group C	PVY^O	PVY^n	PVY^C
P. floridana	M or Mo	N, St	M, St	N	Mo	N
N. tabacum 'White Burley'	M	M	M	Mo	N	Mo
S. tuberosum *'Duke of York'*, (detached leaves)	LL	LL	LL	–	–	LL

N = necrosis, M = mosaic, Mo = mottle, St = stunt

Potato Virus Y

In the past, this virus was generally not considered very important on tomato, because of the mild symptoms produced such as mottle and veinclearing of the leaves. It has been reported on tomato from North America, South America, Europe, Asia and Australia[13,14,15,16,19,20,24,33,35,37,38,51,53,54,55]. However, it is only in Brazil and Australia, that PVY has been considered

a threat to tomato production, because of its frequent occurrence and the considerable yield losses associated with its presence.[3,37,38,51,53]. In both countries, breeding for resistance has been initiated. In Brazil, where several strains of the virus, including one that produces necrosis on tomato, have been recognized, tomato cultivars resistant to both PVY and TMV have already been developed[38]. In southern Europe, the need for breeding PVY resistant tomatoes has lately also been recognized, because of the high incidence of this virus in recent years[35]. Commercial seed companies are so far not yet engaged in breeding PVY resistant tomatoes.

In Taiwan, PVY was first isolated and purified from tomato in 1979[54]. Later, from 1983-1985 a survey was conducted in Tainan and Kaohsiung, the two major tomato production areas and also in Changhua, Nantou and Pingtung to determine the frequency and distribution of PVY. Incidence of PVY in Taiwan was found to be as high as that of CMV[4]. The virus was recovered from each of the surveyed areas and in 34% of a total of 1,608 leaf samples collected. Frequently, plants were found to be simultaneously infected by CMV and PVY. Twenty-eight pure PVY isolates have now been obtained and are being subjected to strain-typing. The symptoms produced on three differential hosts *Physalis floridana*, *Nicotiana tabacum* 'White Burley' and *Solanum tuberosum* 'Duke of York', which are used to distinguish the three common potato strains of PVY, are shown in Table 6. On the basis of symptoms on *Physalis floridana* the PVY isolates from tomato can be classified into two groups, Group B isolates resembling the PVY^C strain, and Groups A and C isolates which do not seem to belong to the three most common potato strain groups of PVY.

Further grouping of these isolates is now underway on several *Capsicum annuum* cultivars. We have noted differences among the isolates in symptom development on *Capsicum annuum* cultivars Yolo Y and Florida VR-2, suggesting that different pathotypes may be present, similar to those described by Gebre Selassie[20] for pathotypes of potato virus Y from sweet pepper and tomato.

A search for sources of resistance in the AVRDC tomato germplasm collection has been initiated in 1984, using PVY isolate 1103 which belongs to Group C. Because of the mild symptoms produced on tomato, we have developed a screening method which enhances symptom expression and facilitates the evaluation and rating. It consists of two inoculations, the first of the newly expanded primary leaves when they are just expanded, and a second one of the newly emerging side branch, after cutting of the stem at the three leaf stage.

We have so far screened a random group of 92 *L. esculentum* accessions and its wild relatives and 16 *Lycopersicon* species all reportedly resistant to PVY. Of these, only one line, *L. hirsutum* PI 247087 was found resistant to the PVY isolate used in the screening[4]. This line is also reported resistant to a PVY isolate from Australia[53].

Crosses between this line and susceptible *L. esculentum* are now being made to study the inheritance of resistance and to develop breeding strategies to incorporate resistance to PVY into AVRDC's heat tolerant tomato lines.

Leafcurl Virus

Leafcurl virus was first reported on tomato in Taiwan in 1983[20]. The symptoms of the leafcurl infected plants, which were found in Tainan County in southern Taiwan were stunting, mild yellowing and reduced leaf size. In leaf squash preparations gemini-type particles of approx. 30 nm in diameter were found[3,22].

The virus could be transmitted by grafting and by whiteflies, but not by mechanical inoculation. Whiteflies transmitted the virus after a minimum acquisition feeding time of one hour on an infected plant and a latent period of three hours in the vector. One whitefly was sufficient for transmission. The host range which was determined by grafting and whitefly transmission included *Datura stramonium*, *Petunia hybrida*, *Physalis floridana*, *Solanum melongena*, *Solanum*

tuberosum and *Lonicera japonica*.

We have attempted several methods of purification that have been used for the isolation of other geminiviruses, but none have been successful so far. Comparative serological tests[40] indicated that the Taiwan tomato leafcurl virus is related to the Japanese tomato yellow dwarf virus, another geminivirus, which causes stunt, yellowing leafcurling and reduction of leaf size of tomato in Japan[39,40].

The short latent period of three hours in the whitefly vector, which is very similar to that of the Japanese tomato yellow dwarf virus, distinguishes the Taiwan tomato leafcurl virus from another whitefly-borne geminivirus, tomato yellow leafcurl virus (TYLCV) which is very destructive on tomato in the Near East and Africa. TYLCV has a latent period of more than 20 hours in the whitefly vector[39].

Active programs for resistance to the tomato yellow leafcurl virus have been in operation mainly in those countries where this virus is endemic and also in France[29,34,45].

Cultivars tolerant to this virus have been developed, using two wild species, *L. pimpinellifolium* Line LA 121[45] and *L. peruvianum*[29,33]. The tolerant character of Line LA 121 is controlled by a single incompletely dominant gene and is expressed by a long latent period of four weeks of the virus in the plant, slow symptom development and mild symptoms. The tolerance, which has been introduced into commercial cultivars is sufficient to permit good fruit set and growth under high vector population and high presence of virus in the field. These commercial cultivars however, are not yet available for distribution outside of Israel where they have been developed.

A better source of tolerance has recently been derived from crosses of two sources of *L pervianum* which are completely symptomless carriers of the virus[29]. Infected plants do not produce any symptoms within four months after inoculation. Highly tolerant materials from interspecific crosses developed by Makkouk and Laterot are now available for field testing.

No information is available, as to whether those TYLCV tolerant cultivars are also tolerant to those geminiviruses, causing leafcurl symptoms on tomato in Asia. We have obtained one such cultivar, LATYLC which will be screened for resistance to the Thailand tomato leafcurl virus through the AVRDC Thailand Outreach Program and to the Taiwan tomato leafcurl virus at AVRDC. At AVRDC, screenings will have to be conducted by grafting to leafcurl infected *N. benthamiana* and *L. esculentum* under controlled greenhouse conditions. Field screening is not feasible in Taiwan because the virus occurs only very infrequently here.

Unidentified Poty Virus

In surveys for tomato viruses, several poty virus isolates were recovered which have not so far been identified[4].

The isolates were transmitted by aphids in a non-persistant manner. The host range was identical for all isolates and was mainly confined to the Solanaceae family including *Datura metel, D. stramonium, Lycium chinensis, Lycopersicon esculentum* 'GRR', 'Delissa', 'Perou', 'CSTMW-18', 'Bonnie Best', *L. pimpinellifolium* LA 121, Univ. of Missouri Acc. 160, *Nicandra physaloides, Nicotiana benthamiana, N. clevelandii, N. debneyii, N. glutinosa, N. sylvestris, N. tabacum* 'Xanthi', 'Xanthi N.C.', 'White Burley', 'Samsun NN', *Petunia hybrida* 'Grandifolia Minstrel', 'Pink Cascade' and *Solanum nigrum*. All isolates produced systemic infection in these hosts. Only two local lesion hosts have been identified so far: *Ocimum basilicum* and *S. tuberosun* 'Duke of York' (detached leaves).

The following hosts are immune to this virus: *Beta vulgaris, Brassica juncea, Capsicum annum* 'Delray Belle', 'Florida VR-2', 'Yolo Y', 'Yolo Wonder', *C. frutescens* 'Tobasco', 'Greenleaf Tobasco', 'McIlhenny', *Chenopodium amaranticolor, C. quinoa, C. murale, Cucumis sativus, Gomphrena globosa, N. tabacum* 'V-20', *Physalis peruviana, Pisum sativun* 'Perfected Wales', *Solanum demissum* x *S. tuberosum* 'A-6', *Solanum melongena* 'Pingtung Long', *S. tuberosum*

'Cardinal', 'Kennebec', *Tetragonia expansa, Vicia faba* minor, *Vigna unguiculata* 'Black' and *Zinnia elegans* 'Bright Scarlet' and 'Tetra Ruffled Jumbo'.

In ISEM serological tests, the isolates were trapped by antisera of poty viruses commonly known to infect tomato or other Solanaceous crops, such as tobacco etch virus, Perou tomato virus, pepper mottle virus, pepper mild mottle virus, pepper veinal mottle virus, potato virus A, wild potato mosaic virus, Columbian datura virus, henbane mosaic virus. It was also not trapped to turnip mosaic virus[4]. On the basis of host range and serological tests, it is believed that this group of isolates represent a new virus so far not described on tomato.

The importance of this virus on tomato in Taiwan is presently being assessed. Preliminary findings indicate that it may be of minor importance only. Of the economically important crops grown in Taiwan, it only infects tomato and tobacco. On the two major hosts, tomato and tobacco, symptoms such as mottle and vein-clearing are mild. Furthermore, in a survey of several tomato growing areas in Tainan, Kaohsiung, Pingtung and Nantou Counties (using ELISA and antiserum to isolate 697), the virus was detected in only 4.85% of a total of 557 leaf samples tested. The virus is also not seed-transmitted.

A search for sources of resistance in *L. esculentum* and wild species is ongoing, but because of the relative insignificance of this virus on tomato, a program for incorporating resistance into AVRDC's heat tolerant tomato lines is not considered at the present time.

Other Viruses

Tobacco etch virus (TEV) and potato virus X (PVX) have been reported from tomato[54] but do not occur frequently[1].

VIRUSES OF CHINESE CABBAGE

Only TuMV[3,8,21,23,31,32] and CMV (Lin, C. C. personal communication) were found in a survey conducted for viruses occurring in *Brassica* crops including Chinese cabbage in Taiwan. The other viruses, commonly reported to infect *Brassica* crops in temperate countries[5,26,27], such as cauliflower mosaic virus (CAMV), radish enation mosaic virus (REV), turnip yellow mosaic virus (TYMV) and turnip crinkle virus were not detected.

Turnip Mosaic Virus (TuMV)

Turnip mosaic virus has been reported from Chinese cabbage in Taiwan as early as 1944[1]. Intensive electronmicroscopic studies were conducted by M. J. Chen in 1975[8]. Following comparative host range studies, conducted with six TuMV isolates originating from radish, *peitsai*, cauliflower, sprouting broccoli, cabbage and leafy mustard, TuMV isolates from Taiwan were differentiated into two groups, Group A which produces virulent reactions on common cabbage and kale but not on radish and Group B which reacted in the opposite way[32]. When Provvidenti's differential set of nine Chinese cabbage cultivars for the identification of different strains of this virus became known[47], attempts were made by researchers at AVRDC and Taiwan Agricultural Research Institute (TARI) to characterize the different strains on this island[21,23,31].

At AVRDC we had already suspected the presence of several strains when we observed an apparent loss of resistance of Chinese cabbage germplasm and breeding lines previously found to be resistant to TuMV. The presence of field strains of TuMV, other than the strain used in the screening tests, was suspected[3]. This and Provvidenti's finding of strain specific resistance in Chinese cabbage prompted us to conduct an island-wide survey to detect as many of the strains of TuMV as possible and use them for the development of TuMV resistant cultivars.

We consequently found TuMV present in all of the nine major vegetable production areas surveyed[21,23]. The virus was recovered from cabbage, Chinese cabbage, cauliflower, broccoli, kohlrabi, radish and leaf mustard plants showing mosaic, mottle, black pinpoint spots, sometimes

ringspots, and also from symptomless plants. All our isolates produced mild symptoms on *N. glutinosa* and on *B. campestris* subsp. *capitat*, indicating that they all belonged to the common strain group which occurs worldwide, but particularly in Asian countries, and not to the cabbage strain group which is confined to Europe, America and Australia[23]. All four strains described by Provvidenti were detected, with strain 4 being the most prevalent strain[21,23]. Strain TuMV-C1 was detected only in one sample. This clearly differs from the situation described by Provvidenti in North America where strains TuMV-C1, TuMV-C2 and TuMV-C3 are most widespread[48]. In addition, a fifth previously undescribed strain was found to be present in Taiwan[21,23]. This new strain, tentatively named TuMV-C5, was isolated from five Chinese cabbage and mustard samples collected in southern Taiwan. In its biological, physical and serological properties, this new strain cannot be clearly distinguished from the other four strains. However, consistently lower ELISA absorbance values were observed with strain C-5 than with the other strains[23]. The results of recent screenings of AVRDC Chinese cabbage accessions and breeding lines for resistance to the five strains indicated that resistance to TuMV-C1 is found frequently in Chinese cabbage. Resistance to C-2 and C-3 has so far been found only in a few lines. However, resistance or immunity to C4 and C5 seems to be rare in Chinese cabbage. So far we have been able to identify only one line, AVRDC accession 730, an F_1 hybrid originating from Korea, with immunity to all five strains of TuMV[4,23]. This line did however, include a small percentage of individual plants susceptible to TuMV-C5. Later F_2's of this accession were found to be impure with respect to their reaction to all five strains except C-3. The resistance mechanism to the five strains in Acc. 730 is presently under investigation to determine the mode of inheritance of the genetic factor(s) involved and assess their potential for use as a source of resistance for AVRDC's Chinese cabbage improvement program. Preliminary studies suggest that resistance to TuMV is partially or incompletely dominant and that resistance to the five strains is not controlled by a single gene.

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VIRUS DISEASES OF SWEET POTATO IN TAIWAN

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SUMMARY

Sweet potato feathery mottle virus, sweet potato latent virus, and a whitefly-transmitted sweet potato virus have been identified in Taiwan. These viruses often occur together in nature. The local lesion transfer is an efficient way to isolate them from the complex. These viruses are readily distinguishable by their symptomatology, host range, transmissibility and stability in saps. The causal agents of two other diseases, sweet potato leaf curl and sweet potato mosaic, have not been identified. Leaf curl or disease complex involving leaf curl caused significant yield losses. Effects of the whitefly-borne virus have not been determined yet.

摘　要

在台灣感染甘藷的病毒已鑑定的有sweet potato feathery mottle virus, sweet potato latent virus和一種粉蝨傳播的病毒，在自然狀態這些病毒常複合感染甘藷，不同的病毒可藉*Chenopodium quinoa*上產生的單一局部病斑來加以分離。這三種病毒可藉著感染甘藷的病徵、寄主範圍，傳播方式和在汁液中的穩定性加以區別。另外兩種病害，甘藷捲葉病和甘藷嵌紋病。其病原毒素尚未分離鑑定出來。甘藷單獨感染捲葉病或遭受包括捲葉病在內的複合感染，均會造成顯著的產量損失。

摘　要

タイワンではsweet potato feathery mottle virus, sweet potato latent virus とコナジラミ傳搬性のsweet potato virusが同定された。これらのウイルスは圃場では重複感染していることが多い。重複感染したウイルスを單離するにはlocal lesionによる分離がよい。病徵、寄主範圍、傳搬性や汁液中の安定性などでこれらのウイルスを容易に區別できる。sweet potato leaf curl 病及びsweet potato mosaic病の病原體はまだ同定されていない。leaf curl 病やleaf curl病と他のウイルスとの重複感染が收量を顯著に減少させる。

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INTRODUCTION

Sweet potato is an important feed and subsidiary food crop in Taiwan, with a total acreage of 36,000 ha[1]. The cultivation of sweet potato is scattered all over the island, and is of special importance in areas where irrigation is limited.

The occurrence of virus diseases of sweet potato was first noticed in Taiwan in 1972[23]. In a general survey, Liao (1978) observed suspect virus and virus-like symptoms in 280 out of 320 varieties in the varietal collection at the Chiayi Agricultural Experiment Station (CAES).

The survey was followed by a series of research efforts to characterize and identify those viruses which were encountered, to derive virus-free clones of major commercial varieties from meristem culture and to determine the effects of different viruses on sweet potato yield and quality[5,6,13,14]. The present article reviews the state of our knowledge of sweet potato virus diseases in Taiwan.

SWEET POTATO VIRUSES DETECTED IN TAIWAN

Three sap-transmissible sweet potato viruses have been reported under the designations of SPV-A, SPV-N and whitefly-borne virus disease. The first two are referred to in this article as the sweet potato feathery mottle (SPV-A) and sweet potato latent virus (SPV-N) respectively, and are wide spread; while the whitefly-borne virus is found only occasionally[8].

Sweet Potato Feathery Mottle Virus (SPFMV)

This virus originally designated as sweet potato virus A (SPV-A) was isolated from sweet potato cultivar Tainung 63[5,14]. It causes systemic interveinal chlorotic spots on Tainung 63 and typical feathery mottling on *Ipomoea nil*. Roots of infected Tainung 63 do not develop russet crack or internal cork. Virus particles are flexuous rods 850-900 nm in length and with a coat protein of molecular weight (Mw.) 37,600. The virus was easily transmitted by sap inoculation or by green peach aphid (*Myzus persicae* Sulzer) and cotton aphid (*Aphid gossypii* Glover) in a non-persistent manner. No transmission was obtained, using corn aphid (*Rhopalosiphum maidis* Fitch) as vector. Seeds of virus infected *I. nil, I. setosa* and sweet potato did not transmit the virus. The host range is restricted in Convolvulaceae and Chenopodiaceae. In *I. nil* sap, the dilution end point of the virus was between 10^{-3} and 10^{-4}, the thermal inactivation point was between 55° and 60°C, and the longevity *in vitro* was less than 24 hrs at room temperature. Three characteristies are consistent with SPFMV described by other investigators[3,4,11,19,20]. This virus has been further identified as a strain of SPFMV by serologycial test. It reacted positively with antiserum against SPFMV-common strain.

The discrepancy in root symptoms may possibly be due to different cultivars or virus strains tested[3,19,20].

Sweet Potato Latent Virus (SPLV)

This virus originally designated as sweet potato virus N (SPV-N) was isolated from Tainung 63 having mixed infection with SPFMV[5,14]. It did not cause symptoms in most potato varieties. Virus particles are flexuous rods 700-750 nm in length with a coat protein of Mw. 36,000. The virus was sap transmissible. No vector has been found. All transmission experiments using *M. persicae, A. gossypii, R. maidis* and *Bemisia tabaci* as vector gave negative results. Seeds harvested from infected *I. nil* and *Nicotiana benthamiana* did not transmit the virus. The host range was mainly limited in Convolvulaceae and Chenopodiaceae, but some Nictoiana species were susceptible to this virus[14,15]. In *N. benthamiana* sap, the dilution end point was between 10^{-2} and 10^{-3}, the thermal inactivation point was between 60° and 65°C, and the longevity *in vitro* was less than 24 hours at room temperature. SPLV did not react with antiserum against FMV-C. The protein fractions prepared from SPLV-infected *N. benthamiana* by low speed centrifugation[2,24], contained tyicpal cytoplasmic-inclusion protein and nuclear-inclusion protein as revealed by SDS-polyacrylamide gel electrophoresis.

SPLV appeared to be distinct from all sweet potato viruses reported so far[3,10,18,21]. It resembled the sweet potato mild mottle virus (SPFMV) in that both caused mild to symptomless infections in sweet potato[10]. However, there are some major differences. Unlike SPFMV, SPLV was not transmitted by whitefly. Moreover, SPFMV caused vein clearing, leaf curling leaf curling and distortion on *N. glutionsal*[10] which was not the host of SPLV[14,15].

A Whitefly-transmissible Sweet Potato Virus

This virus was isolated from Tainung 63 showing foliage symptoms of mottling, chlorosis and dwarfing[8]. Symptoms were more striking at low fertilization conditions or at low temperatures. The diseased plants had poor root systems and produced unmarketable fleshy tubers. The chlorophyll content of virus-infected sweet potato leaves was about 50% less than that of healthy leaves[8].

Virus particles are flexuous rods 750 nm in length with a coat protein of Mw. 33,000. The virus could be transmitted by mechanical inoculation with sap and by the whitefly *B. tabaci* (Chung *et al.* 1985). For successful transmission the vector insect needed an acquisition feeding of 8 hr or longer and the ability of transmission persisted up to 9 days. Attempts to transmit the virus by green peach aphids were unsuccessful. The hosts of this virus included species mainly in Convolvulaceae and Chenopodiaceae, but *Gomphrena globosa, Sesamum orientale, Datura stromonium* and *Cassia occidentials* were also susceptible. In sweet potato leaf sap, the dilution end point was between 10^{-6}-10^{-7}, the thermal inactivation point was between 85° and 90°C, and the longevity *in vitro* was longer than 7 days. The formation of cytoplasmic inclusions in the infected sweet potato leaves was elucidated by the ultrastructural study[8].

SWEET POTATO DISEASES WITH UNKNOWN CAUSAL AGENTS

Sweet potato leaf curl and sweet potato mosaic are two sweet potato diseases with pronounced symptoms found in Taiwan. Attempts to isolate the leaf curl agent were unsuccessful. Information on sweet potato mosaic is still very limited.

Sweet Potato Leaf Curl

A disease showing leaf crinkling and upward rolling symptoms was observed on Tainung 63 sweet potato plants[9,14]. The symptoms were prominent on young infected plants or shoots in

the summer time only and became masked in other months. The disease agent could be transmitted from infected Tainung 63 to sweet potato and *I. nil* by whitefly *B. tabaci* or by grafting. Successful transmission was obtained only when 20-100 insects were used per test plant after 24 hr or longer acquisition feeding.

The host range of the disease agent was limited in Convolvulaceae[9]. Attempts to isolate disease-related proteins and dsRNA, and to purify the disease agent have been unsuccessful so far. Ultrastructural studies revealed short rod-shaped particles approximately 18 nm in width present in the cytoplasm of phloem cells[9]. However, these were indistinguishable from P-protein in normal cells.

Apparently, a similar disease was also reported in Japan[22]. No research findings have confirmed the viral nature of sweet potato leaf curl.

SWEET POTATO MOSAIC

Sweet potato mosaic in Taiwan appears to be distinct from that reviewed by Martin (1957). The disease causes leaf mottling, rugose, dwarfing and distortion. Infected plants have shortened internodes and retarded growth. Sprouts from fleshy roots of harvested infected plants appeared normal in the hot season but showed symptoms in the cooler months. Only a few sweet potato varieties, including Okinawa 100 were found to show mosaic symptoms. This disease is now of minor economic importance because farmers have stopped growing these varieties. The disease incidence was very low in the field. The mosaic symptom could be induced to *I. setosa* by grafting, but in others we were unable to reproduce the disease symptoms by grafting diseased *I. setosa* to healthy sweet potatoes.

EFFECTS OF VIRUS DISEASES ON THE YIELD AND QUALITY OF SWEET POTATO

Screenhouse and field experiments to compare healthy and virus-infected sweet potatoes were carried out. The healthy stock were virus-free, meristem tip cultured sweet potatoes grown under a program initiated in 1978 in CAES[13, 14]. In a field test, the fleshy root yields of cultivar Tainung 57, Tainung 63, Okinawa 100 and *Hong-hsn-wei* infected by a virus complex were 24.5, 35.6, 30.5 and 31.8% less than those of healthy controls[7].

In a field trial to compare the virus-free plants with those of individual and mixed infection by SPFMV and SPLV, Liao *et al.* (1983) observed no significant differences between healthy and virus infected plants in crude protein, soluble sugar and starch content in the roots and tops on a dry matter basis. Yields of both roots and tops were neither affected by infection with SPFMV or SPLV alone nor by a complex infection of both viruses. However, yield loss of fleshy root has been commonly observed in growers' fields.

To investigate the possible effects of sweet potato leaf curl disease (Lc) on yield, virus-free Tainung 63 was inoculated with Lc alone or in complex with SPFMV and SPLV. The results indicated that the yield of sweet potato was considerably reduced if the plant carried Lc.

Effects of the whitefly-brone virus have not been determined yet. Obviously, it appeared to be the most detrimental disease on sweet potato in Taiwan.

DISCUSSION

Some major cultivars of sweet potato have been progapated by farmers for many years without renewal on this island. Vegetative propagation provides a highly efficient mechanism for the perpetuation and dissemination of virus diseases. The high incidence of virus diseases was seen in a survey made by Liao who examined 320 varietal collections at CAES, and observed virus symptoms on 280 varieties. Chung found about the same magnitude of the disease problem when she indexed sweet potato plants from commercial plantings by grafting on *I. nil* or *I.*

sctosa, 86% of the test plants revealed positive reaction.

Among the three filamentous viruses identified by us, SPFMV apparently is a member of potyvirus. Both SPLV and whitefly-borne virus resemble potyvirus in particle morphology and in inducing the formation of cytoplasmic inclusions within tissue of infected plants, but none were transmitted by aphids[9,15]. The taxonomy of these two viruses is therefore uncertain. The failure to demonstrate the aphid transmissibility of SPLV could be attributed to the intrinsic property of the virus itself, the need for a 'helper' virus or the loss of aphid transmissibility evolutionarily. Comparisons of some properties of SPFMV, SPLV, whitefly-borne virus and SPLc with other known viruses of sweet potato are listed in Table 1. Further information such as biochemical properties and serological relationship are needed for classifing these viruses.

Since the whitefly-borne virus infected plants and plants with mosaic symptoms produce sprouts which appear abnormal, growers usually discard them during planting. However, SPFMV, SPLV and the leaf-curl diseased plants might easily be overlooked and used in field plantings. Plants derived from the meristem tip culture which have passed repeated indexing should be the source of stock plants for renewal planting.

Although the healthy controls used in field trials were not resistant cultivars, most of them remained symptomless at the time of harvesting[7,17]. This suggested a slow natural spread of sweet potato virus diseases under Taiwan's conditions. A high degree of sweet potato virus disease control should be achievable by a combination of virus-free seedlings and certain cultural methods, such as planting in the field with low inoculum potential and avoiding the period of high vector population.

Table 1. Comparison of some properties of SPFMV, SPLV, W-SPV[a] and SPLc with other known viruses in sweet potato

Properties	SPFMV	SPLV	W-SPV	SPLc	SPFMV-C[b]	SPMMV[c]
Transmission						
Mechanical means	+	+	+	–	+	+
Aphid	+	–	–	–	+	–
Whitefly	–	–	+	+	–	+
Host range						
Amaranthaceae	–	–	+	–	–	+
Chenopodiaceae	+	+	+	–	+	+
Compositae	–	–	–	–	–	+
Curcurbitaceae	–	–	–	–	–	–
Convolvulaceae	+	+	+	+	+	+
Solanaceae	–	+	–	–	–	+
Properties *in vitro*						
DEP	10^{-3}–10^{-4}	10^{-2}–10^{-3}	10^{-6}–10^{-7}		10^{-3}–10^{-4}	10^{-2}–10^{-3}
TIP (25°C)	55-60	60-65	85-90		60-65	55-60
Aging (day)	1	1	7		1	3-7
Particle length (nm)	850-900	700-750	750		800-850	950
Coat protein (Mw.)	37600	36000	33000			

a) whitefly-borne sweet potato virus
b) reported by Moyer and Kennedy (1978)
c) reported by Hollings *et al.* (1976)

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DISCUSSION

Q. (N. Namba)
In Japan Dr. Yamashita succeeded in isolating small bacilliform virus particles from leafcurl diseased sweet potato and whitefly inoculated test plants. Have you investigated this?

A. We have not had much progress in isolating the pathogen from leafcurl diseased sweet potato or any other hosts.

Q. (N. Namba)
In your work have you been able to observe such bacilliform particles under electron microscope?

A. No particles were found in quick leaf-dip preparations of infected sweet potato. Electron microscopy of ultrathin sections of diseased sweet potato revealed that virus-like, rod-shaped particles, approximately 18 nm in width were present in the cytoplasm of phloem cells. However, it was indistinguishable from P-protein in normal cells. This needs to be further investigated.

DASHEEN MOSAIC VIRUS AND ITS CONTROL IN CULTIVATED AROIDS

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SUMMARY

Dasheen mosaic virus, a potyvirus initially described in 1970, infects cultivated aroids throughout the world. Technological advances have made the control of this virus possible through seed propagation and/or tissue culture. However, to apply these control measures commercially, a careful evaluation of the horticultural advantages to be gained and the costs involved must be given for each aroid in question.

摘 要

Dasheen 嵌紋病毒爲1970年首次報告的一種絲狀(Potyvirus)病毒，普遍存在各地，感染栽培的天南星作物。在技術上，利用種子繁殖或組織培養的方法，可以控制此一病毒。但要能實際的應用到商業上，必須謹慎的評估其在園藝上能獲得的利益，以及每一種天南星在操作時所需之費用。

摘 要

1970年に初記載されたPotyvirusに屬するサトイモモザイクウイルスは世界中のサトイモ科植物の栽培種に廣く分布している。種子繁殖または組織培養によつてこのウイルスを防除する技術はできた。しかし、これらの防除法を實用化するためには、それによつて得られる園藝上の利益とその費用について問題となつている作物それぞれについて注意深く評價する必要がある。

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INTRODUCTION

The family Araceae comprises more than 100 genera and 1500 species including one, *Pistia stratiotes*, which is an important waterweed. Most aroids are beneficial however, and some are of considerable significance economically, particularly in the tropics. Two, *Colocasia* and *Xanthosoma*, are especially important as food staples both in the new and old world, although *Amorphophallus*, *Cyrtosperma*, and *Alocasia* are also used for this purpose in certain locales. Aroids such as *Aglaonema*, *Caladium*, *Dieffenbachia*, *Philodendron*, *Scindapsus*, and *Syngonium* account for about a third of the foliage plants grown, certain species of *Anthurium*, *Richardia*, and *Zantedschia* are important cut flower crops, and *Cryptocoryne* is one of the most widely used aquarium plants. Most cultivated aroids, including all the aforementioned, are routinely propagated vegetatively, and as such are especially vulnerable to chronic virus infections.

VIRUS DISEASES

Several viruses and virus-like diseases are known to infect aroids. These include tobacco necrosis virus of *Dieffenbachia*[47], cucumber mosaic of *Arum* and *Colocasia*, tomato spotted wilt of *Zantedeshia*, and the bobone rhabdovirus of *Colocasia*[16,56]. Other less well defined reports include the banana bunchy top[50] and "almoae" diseases of *Colocasia*, the "chirke" disease of large cardomon, which infects *Acorus, and unnamed* viruses of *Anthurium*, Monstera, Philodendron, *and Zantedeschia*[60,77]. None of these viruses, however, infect as many aroids or is as wide spread as dasheen mosaic virus (DMV), a potyvirus first described in 1970. This virus occurs throughout the world and infects at least 14 aroid genera: *Alocasia, Aglaonema, Amorphophallus, Anthurium, Arisaema, Caladium, Colocasia, Cryptocoryne, Dieffenbachia, Philodendron, Richardia, Spathiphyllum, Xanthosoma,* and *Zantedeschia.* Although certain nonaroids are susceptible, the natural host range of DMV appears to be restricted to the family Araceae[52,77].

Dasheen Mosaic Virus

Dasheen mosaic virus symptoms may differ considerably according to the aroid infected and the season in which it is grown[77]. In some aroids such as *Richardia, Zantedeschia,* and certain *Dieffenbachia* cultivars, symptoms can be quite severe, whereas in others, such as *Aglaonema* and *Spathiphyllum,* they are usually much less evident. In many colocasoid aroids (Tribe Colocasiae), including *Caladium, Colocasia,* and *Xanthosoma,* conspicuous "feathering symptoms" are typical[2,8,12,13,53,56,76]. A characteristic of many aroids is that DMV symptoms are intermittently expressed, often making detection difficult[6,47,66,73,77]. In some instances, such as with *Dieffenbachia,* symptom expression is seasonal, most often appearing on foliage produced during the fall and/or spring months[9,18,52,54,77]. Some aroid cultivars more readily express DMV symptoms than others. The *Caladium* cultivars, 'Candidum' and 'White Christmas', for example, are much more likely to exhibit symptoms throughout the growing season than the cultivars

'Frieda Hemple' and 'Carolyn Whorton'. Similarly, under the same greenhouse conditions, plants of the Hawaiian *Colocasia* cultivar, 'Mana Lauloa', produced leaves with DMV symptoms much more frequently than a cultivar grown in north Florida USA and locally referred to as "dasheen".

The Properties of DMV have been summarized previously[77]. Like other potyviruses, DMV has a mean particle length ca. 700-800 nm, induces cylindrical inclusions, and is transmitted by aphids in a stylet-borne manner. Also, its capsid protein is serologically related to those of certain other potyviruses, including blackeye cowpea mosaic, tobacco etch, and araujia mosaic[29,77]. The cylindrical inclusion protein of DMV is serologically related to that of the araujia mosaic potyvirus[29], and the helper component protein of tobacco vein mottling potyvirus is related to *in vitro* synthesized gene products of DMV[31]. About 93% of the estimated coding capacity of the DMV gene has been accounted for, and the proposed gene map for DMV is similar to that determined for other potyviruses: 5' end-proposed helper factor protein-cylindrical inclusion protein-unknown protein-47K nuclear inclusion protein-56K nuclear inclusion protein-capsid protein-3' end (J. Nagel & E. Hiebert, unpublished).

Aside from the characteristic symptoms it induces, DMV can be diagnosed in a variety of ways, including bioassay, serology, and/or by light and electron microscopy. Bioassay has been widely used, usually involving *Philodendron selloum* seedlings as assay plants[77]. Seeds of this plant can be commercially obtained in the United States, and they are extremely susceptible to this virus, giving mosaic and vein clearing symptoms on the first 1-2 leaves expressed following inoculations. Other aroids such as *Anthurium* spp. can also be used when seedlings of *P. selloum* are not available[2,53,54,54,77]. A limitation in the use of *P. selloum* and other araceous assay plants is that seeds do not retain their viability long[25,62]. Local lesion hosts exist, including the aroid, *Philodendron verrucosum*[67,73], and the non-aroids, *Chenopodium* spp.[52] and *Tetragonia expansa* (A. A. Brunt, personal communication). However, these are not necessarily satisfactory for indexing purposes. *Philodendron verrucosum* is not readily available either as seed or vegetative material, and because it is a rather large plant, it may be inconvenient to maintain under greenhouse conditions. The susceptibility of non-aroids appears to vary according to where and under what test conditions the work is being conducted. For example, in contrast to European studies[52] (A. A. Brunt, personal communication), attempts at Florida USA[76] and Venezuela[12] to infect non-aroids have not been successful.

Since DMV is the only ascertained potyvirus to infect aroids[42], detection of cylindrical inclusions either by light or electron microscopy can be taken as circumstantial evidence for its presence[77]. However, the possibility of previously unidentified potyviruses infecting aroids can not be discounted, and thus some caution should be exercised in making specific diagnoses of DMV by these methods. Should other aroid potyviruses be found, morphological differences in the inclusions induced may be found which may be of diagnostic value, as has been shown for other potyviruses[11,30]. Likewise, since it is the only definitive flexuous-rod virus known to infect aroids, the detection of such virus particles by electron microscopy can also be used as evidence for DMV. However, the same precautions noted above for inclusions should be taken. Any doubts in this regard can be eliminated, however, through the use of DMV capsid antiserum and immunospecific electron microscopy techniques, such as those used for detecting DMV in *Dieffenbachia*[9,32].

Various other serological methods, using either capsid or cylindrical inclusion antiserum, can be helpful for diagnosing DMV infections[77]. The techniques and immunodiffusion medium described by Purcifull and Batchelor[49] have been frequently used for this purpose as has the immunodiffusion medium of Tolin and Roane[65], which contains 0.8% Nobel agar, 0.2% sodium dodecyl sulfate, 0.7% NaCl, and 0.1% sodium azide. Immunodiffusion methods have been used to demonstrate the presence of DMV in Europe[47].

[52], Latin America[51,53] (J. Escudero, J. Bird & F.W. Zettler, unpublished), Africa[2,14,71], (F. W. Reysenbach, personal communication), the People's Republic of China (F. W. Zettler & J. H. Tsai, unpublished), and California USA[39]. Although not yet widely used for DMV, enzyme-linked immunosorbent assay methods also can be used for diagnosing DMV (unpublished); however, the possibility of the existance of serologically distinct strains of DMV should be considered when using direct double antibody sandwich methods, which are relatively strain specific[6,8]. That different DMV strains may exist has been indicated in studies by Abo El-Nil *et al.*[3] and Wisler *et al.*[73].

Regardless of the diagnostic technique used, virus titer variabilities should be taken into account when negative results are obtained. Systemically infected *P. selloum* seedlings support very high DMV titers shortly after plants are inoculated, but titers appear to drop considerably in successive leaves formed thereafter, despite the presence of conspicuous foliar mosaic and distortion symptoms[3,77]. Similarly, considerable titer differences between symptomatic and asymptomatic tissues of individual *Dieffenbachia* leaves were noted [9]. While definitive quantitative studies have not yet been conducted, some aroids such as *Caladium, Colocasia, Xanthosoma, Zantedeschia,* and certain *Philodendron* and *Dieffenbachia* cultivars in general seem to support much higher virus titers than others, such as *Aglaonema, Anthurium,* and *Spathiphyllum.*

Dasheen mosaic virus appears to be common wherever aroids are extensively cultivated, most commonly infecting *Colocasia, Caladium, Dieffenbachia, Zantedeschia,* and *Xanthosoma.* Prior to 1978, it was known to occur in the Caribbean, Florida USA, Egypt, Europe, Japan, India, and Oceania[77]. Since then, it has been reported infecting 1) *Aglaonema* in California USA[39], 2) *Alocasia* in Brazil[53], *3) Cyrtosperma* in the Gilbert Islands[55], 4) *Dieffenbachia* in Florida USA[9], the Untied Kingdom[32], Denmark[47], and Belgium[54], 5) *Philodendron* in Florida USA[73], 6) *Richardia* in Italy[52], 7) Zantedeschia in the Republic of South Africa (F. W. Reysenbach, personal communication), and 8) *Colocasia* and/or *Xanthosoma* in Brazil[53], the Cameroons[14], Costa Rica[51], the Dominican Republic (J. Escudero, J. Bird, & F. W. Zettler, unpublished), French Polynesia[33], Gilbert Islands[55], Guam (G. Beaver, personal communication), Nigeria[71], Papua New Guinea[56], People's Republic of China[13] (F. W. Zettler & J. H. Tsai, unpublished), and the Republic of South Africa (F. W. Reysenbach, personal communication). Although the virus is widely distributed in aroid plantings throughout the world, exact figures for yield losses are usually not available. However, in studies involving the ornamentals, *Caladium, Dieffenbachia, Philodendron,* and *Zantedeschia,* quantitative yield losses of more than 60% were recorded[10,24,38,73,78].

The cosmopolitan distribution of dasheen mosaic virus can be attributed to several factors. The first is that DMV typically induces chronic rather than lethal infections in their hosts, unlike the alomae disease of *Colocasia,* which is confined to certain locations in Oceania[16,56]. Where DMV does induce lethal symptoms, such as with certain *Dieffenbachia* cultivars (*D.* x *Bausei* and *D.* x *memoria-Corsii*), it is self eliminating, and as a result, surveys of foliage nurseries for DMV-infected plants of these cultivars revealed no infected plants[9].

A second important factor in the wide-spread distribution of DMV is that, like most potyviruses, it is readily transmitted by aphids[15,36,44]. Two aphids, *Aphis gossypii* and *Myzus persicae,* which are known to be vectors of DMV, can be found wherever aroids are cultivated. Another species, *Pentalonia nigronervosa,* apparently cannot transmit this virus, however, even though it is a pest of certain aroids[44]. The rapidity with which DMV infections can spread by aphids under field conditions was illustrated in an experiment conducted on Caladium in Florida USA. A population of DMV-free, tissue culture derived plants were planted adjacent to commercially grown stock, and although the tissue culture derivatives significantly outyielded their diseased counterparts, all tissue culture derived plants were infected within two months after planting[38].

Thirdly, widespread incidence of DMV is abetted by the necessity of propagating aroids vegetatively. Although DMV is apparently not seed-borne[77], obtaining seed poses special problems for most aroids, thereby precluding this approach towards virus control. For *Caladium, Colocasia, Dieffenbachia, Philodendron, Xanthosoma,* and many other aroids, a condition of protogyny exists in which the stigmata are receptive prior to pollen shed. This characteristic prevents self pollination within the same inflorescence. Moreover, the period of time that the stigmata are receptive is very brief, usually a matter of a few hours[25,35,43,69]. Three other problems with the use of true seed for aroids are that 1) many horticulturally desirable aroid cultivars flower infrequently, although this problem can be overcome by treating plants with gibberellic acid[4,19,27], 2) the viability of aroid seed is usually of very short duration[25,62], and 3) the seedling progeny of most cultivated aroids exhibit considerable phenotypic variability since they are not true-breeding[26,63,70,72,74,75]. *Philodendron selloum* is exceptional in that it is routinely seed propagated commercially, and as might be expected, DMV incidence in this species appears to be very low[73].

Finally, the horticultural importance of aroids as food staples and ornamentals has been a major factor in the distribution of DMV throughout the world. Taro (*Colocasia*), for example, is believed to be among the earliest plants to be cultivated. As such, this plant accompanied early explorers throughout the Pacific Basin in prehistoric times, and in Egypt it has been grown perhaps as early as 500 B.C.[48] *Xanthosoma,* an indigene of the neotropics, has also been widely distributed since ancient times. Since then, it has been introduced into many places in the old world, including Equatorial Africa where it is an important food staple[45]. In more recent times, the ornamental aroids have been also internationally distributed. *Caladiums,* native to the Amazon Basin, are highly prized for their attractive foliage and have become a specialty industry in south central Florida USA, where they currently constitute an eight million dollar industry. Similarly, foliage aroids such as *Aglaonema, Dieffenbachia,* and *Philodendron* collectively comprise about 30% of a 300 million dollar industry in the United States. In sharp contrast to the edible aroids, which are not are not widely exported commercially, ornamental aroids are shipped throughout the world in large volume. This transcontinental interchange of foliage plants has been especially apparent in recent years due to the "foliage boom" of the 1970's. The number of foliage plants imported into Florida, for example, rose from 2.8 million plants in 1969-1970 to 136.7 million in 1979-1980, primarily from the West Indes and Central America. In turn, Florida now exports over 10 million foliage plants to over 50 countries throughout the world[57].

A related factor in the spread of DMV and other pathogens has been through international exchange of germ plasm. *Caladium, Colocasia,* and/or *Xanthosoma* germ plasm collections in Hawaii[8], Puerto Rico[6], Venezuela[12], and Florida USA (Zettler, unpublished) have been surveyed for DMV infections, and incidences were very high in all instances. Jackson and Firman[34] have recommended guidelines whereby the international distribution of aroid pathogens through germ plasm collections can be reduced.

Although attempts to eliminate DMV from *Xanthosoma* corms through heat treatment were unsuccessful[5], virus-free plants of several aroids have been obtained through seed propagation and tissue culture[10,20,25,58,69,77]. Also, careful selection and exclusive use of symptomless cuttings for propagating material has been used successfully to control DMV infections of *Dieffenbachia* in Europe[47,54] (Hakkaart, personal communication). In *Dieffenbachia,* DMV appears to be intermittently distributed in infected plants, which makes it possible to obtain healthy plants when care is taken to avoid using cuttings with DMV symptoms[10,78]. Similar observations were made by Wisler *et al.*[73] for *Philodendron oxycardium.* No true genetic resistance to DMV is known, although some cultivars of *Dieffenbachia*[9] and *Xanthosoma*[71] seem to be more tolerant than

others.

CONTROL OF DMV

Theoreticaliy, the use of tissue culture would be the most practical solution for controlling DMV in the majority of cultivated aroids. This approach has proved successful in obtaining virus-free, genetically uniform plants from diseased parental stock and in many instances has the added advantage of being an efficient means of rapid plant propagation. The first aroids to be successfully propagated *in vitro* were *Caladium*[22] and *Colocasia*[41]. Since then, tissue culture methods have been improved and successfully applied to many other aroids, including *Anthurium, Dieffenbachia, Philodendron, Spathiphyllum, Xanthosoma,* and *Zantedeschia,* all of which are susceptible to DMV (among others:[1,7,17,20,28,37,40,46,59,61,64,79]) To date, however, certain other DMV-susceptible aroids have proven much more difficult to culture *in vitro,* such as *Aglaonema.*

Currently, commercial aroid tissue culture is done primarily for puroses of rapid propagation, rather than for the control of DMV per se. Also, tissue culture is largely confined to such foliage aroids as *Dieffenbachia, Philodendron, Spathiphyllum,* and *Syngonium,* which are typically greenhouse rather than field grown. The deployment of tissue culture technology for aroids has been extremely rapid and coincided with the 15-fold expansion of the foliage industry since the early 1970's. Through tissue culture, for example, a single shoot tip of *Dieffenbachia* can produce up to 70,000 cuttings or more in one year[28] compared to only 10-30 cuttings per year by conventional propagation methods. Application of tissue culture technology not only helped to meet the sudden consumer demand for foliage aroids, but it also produced a product of unusual quality and uniformity. *Dieffenbachia,* once apparently ubiquitously infected with DMV[23], is now largely free of this and such pathogens as *Erwinia chrysanthemi* wherever tissue culture derived stock is employed[9,10]. Since these foliage aroids are normally greenhouse-grown, reinfection by DMV can be prevented when usual pest control practices for foliage aroids are employed. Many tissue culture operations have been established in recent years. The largest in Florida are Hartman's Plants Inc., Oglesby Plant Laboratories Inc., and Weyerhaeuser Tissue Culture Center, each with the current capacity to produce 6 to 25 million tissue culture explants annually at an estimated wholesale value of $US 0.10-0.20/explant. Other major tissue culture facilities in the United States are located in California, Tennessee, Texas, and Utah. In other countries, major tissue culture laboratories are located in Belgium. England, France, Israel, Japan, and the Netherlands.

Tissue culture has not been as widely applied for such field-grown aroids as *Caladium, Colocasia,* and *Xanthosoma,* although the potential exists for these crops as well. Hartman[20] demonstrated that these plants could be freed of DMV and that rapid propagation as noted above for foliage aroids could readily be achieved. However, because they are field grown, the problem of reinfection must be considered if a successful control strategy is to be implemented. Fortunately, there are relatively few reservoirs of DMV inoculum. Many aroids occur as weeds, but these tend to be much less common and aggressive than weeds in other plant families, such as the Commelinaceae. As pointed out be Rana *et al.*[52], nonaroid suscepts of DMV are unlikely to be epidemiologically significant since most are local lesion hosts. Accordingly, primary sources of DMV inoculum which could threaten tissue culture derived healthy stock is likely to be solely from diseased plants of the same or related species which have not been processed through tissue culture and are growing nearby. Thus, if conditions of isolation are provided, DMV control can be expected. For example, a 2.4 hectare field of virus free, tissue culture-derived *Caladium* plants isolated by about 65 Km from the nearest diseased commercial stock was manitained free of virus for over three years (R. D. Hartman & F. W. Zettler, unpublished). Similarly, none of over 5000 seed-derived *Caladium* plants, which were isolated from com-

mercial stock in north-Florida, became infected with DMV in a 4-year period[74].

Although tissue culture technology has been refined whereby large numbers of DMV-free *Colocasia, Xanthosoma,* and other edible aroids can readily be produced, special problems must be overcome if this approach is to be applied commercially. One of the most obvious is that the edible aroids tend to have much lower cash values per plant than their ornamental counterparts. Moreover, these crops are usually grown in countries where growers can ill afford to buy the relatively expensive tissue-culture derived stock. Finally, as pointed out by Plucknett[48] for *Colocasia* and Morton[45] for *Xanthosoma,* the edible aroids are an exceptionally diverse group of plants, consisting of innumerable cultivars grown by many different ethnic groups throughout the world, each on relatively limited acreages. To implement a tissue culture program for any given plant, a very large initial expense is needed. The construction of a tissue culture facility requires an initial investment capital of $US 20,000-250,000, depending upon the scope of the operation[21], and modern, up-to-date facilities are likely to exceed to US$ 1,000,000 to construct. In addition, to prepare each new plant for development, an initial expense of $US 2,000-10,000 is required. Obviously then, enough sustained orders must be forthcoming to justify the initial expense involved in tissue culture, and unfortunately this appears not to be the case at this time for most of the edible aroids.

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DISCUSSION

Q. (C.N. Roistacher)
Has heat treatment or thremotherapy been applied to eliminate DMV in the aroids?

A. No, as it has not been necessary.

Q. (D. Gonsalves)
What strategies would be suitable for transferring this technology to developing countries which are dependent on taro as a staple food?

A. It is a question of investment of your time which is important. To apply this in a developing country there are several key points. Number one is that you need the contact people; for example here in Taiwan there are excellent facilities like COA. This is the level of people we need if ever it were to be applied, someone right on the spot. Number two is the area of the crop, Hawaii may be a good place, a large area crop is needed to work on. But in Hawaii there is a massive proliferation of varieties, almost a separate one for each religious festival, where as in Taiwan the problem would be simplified as there are only two main varieties of taro. Number three is how to do it. I would expect that an example would have to be set up, a model experimental plot for the growers to see and encourage them to accept the idea. Then there are the problems of inertia and finally the economics of the crop.

Q. (D. Gonsalves)
Is trying to get a resistant plant a viable approach?

A. We tried that, we got a form of resistance in certain ornamentals, but I do not believe this was resistance to true mosaics. In the ornamentals we can control DMV so easily with tissue culture that it makes no sense to spend research on resistant varieties.

Q. (S.K. Green)
Using these techniques of tissue culture as control, do you see any danger in losing the genetic diversity of these crops; particularly if applied to taro in developing countries?

A. I fell very strongly about genetic diversity. I think what happens in this type of program is that it is accompanied by a search for genetic material as a basic, and though ultimately what the consumer gets may be more limited,the breeding program behind its development may actually have increased the genetic diversity of the basic material.

Genetic diversity, I strongly advocate, particularly as the environment takes away natural habitats. I also know that there is a great danger in the importation of plants because I think that this is how the viruses have become very widespread. It is principal that what the consumer receives is only what he needs, but the breeding stock is increased by collection.

Q. (C.N. Roistacher)
What is the incidence of natural infection? Should one grow virus free material, in native areas where patches of taro are being grown, or alternatively there may be infection from native wild vegetation.

A. It is a matter of proximity, growing aroids in isolation requires an efficient rapid tissue culture program so that the plants can be propagated under field conditions faster than the virus can spread. Aroids are a little easier in this respect than perhaps citrus or cucurbits, because the virus is essentially restricted to the aroids, and the aroids are not agreesive weeds. So in many areas we can isolate them, even in places like Taiwan and Florida, helped by this narrow cultural range.

SECTION III

VIRUSES OF FRUIT CROPS

RECENT RESEARCH DEVELOPMENT IN VIRUS DISEASES OF GRAPEVINES

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SUMMARY

Despite extensive research the etiology of grapevine leafroll disease, the most widespread and serious virus disease of grapevines, remains unclear. Recent research indicates that at least two closterovirus-like particles are associated with the disease. Their identification will enhance prospects for determining the etiology and developing a rapid diagnostic technique for the disease.

摘　要

葡萄捲葉病(GLRV)爲葡萄中最廣泛與嚴重病害，雖有極多之研究，但有關病源則仍未明瞭。目前研究指出至少有二種以上 Closterovirus 族與此病害有關。其經判明後，對於病源將可進一步了解，並有助於快速診斷方法之發展。

摘　要

ブドウのウイルス病のうち最も被害が大きく分布の廣いブドウ葉巻病は多くの研究が行われたにも拘わらずその病原は未だにはつきりしていない。最近の研究によれば、少くとも2種類のclosterovirus様の粒子と本病との關係が認められる。これらの諸性質を明らかにすることにより、その病原が確定し、また迅速な診斷法の開發への道が開かれる。

RECENT RESEARCH DEVELOPMENT IN VIRUS DISEASES OF GRAPEVINES

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INTRODUCTION

Grape is the most widely grown and economically important fruit crop in the world. Like many other fruit crops, however, grapes are affected by numerous diseases, viruses being among the most important. Grapevine leafroll is the most widespread and important virus disease of grapes on a world wide basis[8].

The establishment of vineyards with virus-free vines is the initial and most important control measure for leafroll and other grape viruses. This, in turn, is dependent on having a reliable method for diagnosing the disease. Leafroll and most grape virus diseases are diagnosed by indexing on woody indicators that show characteristic symptoms. These tests have been very useful in detecting virus diseases in symptomless rootstock and scions and have been the foundation on which many grape industries have developed certification programs which have helped to increase crop production. However, several shortcomings are obvious in these tests. First, they take anywhere from one to three years to complete. Second, because of the expense and labor involved with these tests, it is impractical to do large scale indexing to determine the occurrence of the disease in vineyards. Third, since these tests rely on symptom manifestations, it is conceivable that certain strains of leafroll would not be detected by these indicators.

Despite the economic importance of leafroll, the causal agent(s) of the disease has not been identified. This lack of information has prevented the employment of indexing techniques which are more rapid and less expensive than the present ones. For example, the enzyme-linked immunosorbent assay (ELISA) test has proven to be extremely useful for diagnosing numerous fruit tree viruses which previously required the use of long term tests with woody indicators, e.g. tristeza[1]. Clearly, information on the identification and properties of the causal agent(s) of the leafroll disease is a fundamental prerequisite for obtaining a better understanding of the disease, for developing more rapid and reliable indexing procedures, and for developing more efficient methods to control this serious disease of grapevines.

HISTORICAL BACKGROUND ON ETIOLOGY OF GRAPEVINE LEAFROLL DISEASE

Grapevine leafroll is a graft transmissible, virus-like disease for which the etiology has not been demonstrated. Affected grapevines show a downward rolling of the leaves and a reddening of the interveinal leaf surface on varieties with dark-colored fruit. Varieties with light-colored fruit also show rolling of leaves but develop interveinal chlorosis. Economic damage is primarily due to reduced yield over a period of time and to the significantly lower sugar content in grapes from diseased vines. Leafroll can be distinguished by its characteristic symptoms (rolling of leaves and interveinal reddening of leaf) on the woody indicators Mission or Cabernet Franc[8].

A number of investigators have associated virus-like particles with leafroll. These include isolmetric particles[12], filamentous particles resembling a potyvirus[16] and filamentous particles resembling closteroviruses[3,5,6,9,11,13,17]. Recently, a viroid with similar nucleotide homology to hop-stunt viroid was isolated for the first time

from grapevines[15]. However, no effort was made to correlate it to leafroll or any other grape disease. So far, none of the above reports have shown that the observed particles can cause leafroll symptoms on inoculated grapevines.

However, an increasing amount of evidence is accumulating to support the idea that leafroll is caused by one or more closteroviruses. Namba *et al.*[13] first observed closterovirus-like particles in cells and extracts of leafroll infected grapevines but not in healthy vines. Since then, other workers have also observed closterovirus-like particles in the phloem tissue of grapes infected with leafroll[3,6,17]. Another closterovirus, which was originally from a grapevine showing stem pitting symptoms and was subsequently transmitted to *Nicotiana clevelandii*, has been isolated and characterized[2,5]. Although the virus can be mechanically transmitted from *N. clevelandii* to *N. clevelandii*, it has not been transmitted back to grapevines nor has it been transmitted from grapevines to *N. clevelandii* again. The virus, designated as grapevirus A (GVA), is 800 nm long, has a single-stranded RNA genome, and is reported to be transmitted by mealybugs[2,14]. Recently, Milne *et al.*[11] showed that GVA is present in numerous diseased grapevines. Also, they found another shorter closterovirus-like particle, which is serologically distinct from GVA, in many of the grapevines. The association of one or both of these particles with grapevines infected with leafroll was about 50%.

RECENT RESEARCH INFORMATION ON ETIOLOGY OF LEAFROLL DISEASE

Very recently in Switzerland, Gugerli *et al.*[9] showed a close, if not perfect, correlation of the leafroll disease with closterovirus-like particles either 1800 nm or 2200 nm in length. The particles were detected in clarified and concentrated grape leaf extracts from infected but not healthy vines. Antiserum was prepared to the 2200 nm particles and used in ELISA to diagnose the disease in vines known to be infected with leafroll. Since the antiserum did not react with leaf extracts of infected grapevines containing the 1800 nm particles, they concluded that there may exist two strains of closteroviruses associated with leafroll in Switzerland. Although a number of reports have associated closterovirus-like particles with leafroll disease, there are conflicting data (summarized in Table 1) on the length of particles which are associated with the disease. It is conceivable that the leafroll disease syndrome may be separately caused by one or more of these virus-like particles. In fact, the work by Gugerli *et al.*[9] indicated that this may be the case, since they found 1800 nm and 2200 nm particles separately associated with leaf roll disease. Recently, we have isolated closterovirus-like particles from known isolates of leafroll from California and New York[18]. The methods we used were those of Gurgerli *et al.*[9] or a modification of them (unpublished). Studies are underway to characterize the isolated particles.

FUTURE PROSPECTS FOR DETERMINING THE ETIOLOGY OF LEAFROLL DISEASE

Indexing with grape indicator plants is the current accepted method for diagnosing leafroll. The enzyme-linked immunosorbent assay (ELISA) technique[4] would be a good alternative for detecting the disease. The recent work by Gugerli *et al.*[9] is the only published report which uses ELISA for detecting leafroll-infected vines. Antiserum to the 2200 nm particles were used in ELISA to detect the virus in leafroll diseased vines in which the 2200 nm particles were present. Although antiserum has been produced to GVA[5], there are no reports which show that GVA can be detected by ELISA in infected grapevines. We have recently produced an antiserum to one of our leafroll isolates and are testing its usefulness for detecting the virus in grapevines (unpublished).

Given the possibility that leafroll may be caused by more than one type of closterovirus-like particle, it is imperative that specific antisera be produced to these particles. Unfortunately, these

Table 1. Summary of data associating closterovirus-like particles with leaf roll.

Origin	(plant)	Particle length (nm)	Characterization (E.M., isolated)	Antisera reaction GVA	2200
Japan	(grape)	1000	E.M.	NT	NT
Italy	(grape,	800	E.M., isolated	+	NT
	N. clev.)	400(?)	E.M.	–	NT
Germany	(grape)	2000	E.M.	NT	NT
Switzerland	(grape)	1800	E.M., isolated	–	–
		2200	E.M., isolated	–	+

E.M. = electron microscopy, NT = not test, + = positive, – = negative.

different particles have been detected in the same plant[11]. Since the leafroll agent has not been mechanically transmitted there is no way to biologically purify the isolates through mechanical transfer. The use of monoclonal antibody technology[10] would be valuable for selecting antibodies which are specific to each virus type in a heterologous virus population.

There are no published reports on reproduction of leafroll disease by mechanical inoculation (excluding graft inoculation) of grapevines. However, unequivocal evidence on the causal role of closteroviruses (or any other agents) to the leafroll disease will be obtained only when this condition is satisfied. Until then, investigators will be limited to determining the 'association' of virus-like particles with the leafroll disease. It seems that mechanical transmission of leafroll virus may be possible using the "knife slash" method which has been used to transmit tristeza virus[7].

With the isolation and purification from grapevines of at least two types of closteroviruses associated with leafroll, mechanical inoculation experiments can now be done to critically determine the role of these viruses in the leafroll disease. In summary, the prospects are bright for developing more rapid methods for diagnosing leafroll and for determining the etiology of this widespread and serious virus disease of grapevine.

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THREE PHLOEM-LIMITED VIRUSES OF GRAPEVINE: DIRECT FLUORESCENCE DETECTION

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SUMMARY

Grapevine ajinashika virus (GAV), grapevine stunt virus (GSV) and grapevine leafroll virus (GLRV), important in Japanese viticulture, are all phloem-limited viruses. GAV and GSV are small and spherical, ca. 25 nm in diameter. GAV was purified and diagnosed with ELISA. GSV was found to be transmitted by grapevine leafhopper (Arboridia apicalis Nawa; Cicadellidae). Cicadellidae is a new insect vector of plant virus. GLRV is a long, flexuous, rod-shaped particle, approximately 11x1,000 nm, which is a probably a member of the closterovirus group. Infections of these viruses are detectable by the direct fluorescence detection (DFD) method.

摘 要

葡萄無味果病毒(GAV)，矮化病毒(GSV)與捲葉病毒(GLRV)，均屬於靱皮部感染，爲在日本葡萄園中之主要毒素病，無味果病毒和矮化病毒球形，直徑大約25nm，無味果病毒純化後，可用ELISA檢定。葡萄矮化病毒係由葉蝨（*Arboridia apicalis* Nawa;Cicadellidae)傳播，葉蝨爲植物病毒新的一種媒介昆蟲。葡萄捲葉病爲一長線形顆粒，長度約11 × 1000 nm，屬於Closterovirus族。這些病毒感染後，均能以直接螢光方法加以檢定。

摘 要

ブドウ味無果ウイルス（GAV）、ブドウ萎縮ウイルス(GSV)、ブドウ葉巻ウイルス（GLRV）は日本のブドウ栽培にとつて重要なウイルスルであり、すべてし部局在性である。GAVとGSVは直徑約25nmの小球形ウイルスである。GAVを純化しELISAで診斷した。GSVはブドウヨコバイ（*Arboridia apicalis* Nawa; Cicadellidae)，Cicadellidaeは植物ウイルスの新しい媒介昆蟲である。GLRVは長さ約1,000 nm 巾11 nm の長い屈曲したかん狀粒子であり、closterovirus groupに屬するす思われる。これらのウイルス感染は直接螢光検出法(DFD)で検出できる。

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GRAPEVINE AJINASHIKA VIRUS (GAV)

In Japan, GAV, GSV, and GLRV are the most important of grape viruses; other viruses, grapevine corky bark virus and grapevine fanleaf virus etc., cause almost no symptoms on cultivars. Both GAV and GSV are small and spherical, ca. 25nm in diameter. The location of both viruses are restricted to phloem cells (Fig. 1,2)[19,20,21]. Recently, GSV was found to be transmitted by grapevine leafhopper (*Arbordia apicalis* Nawa)[10,17]. GLRV is a long, flexuous, rod-shaped particle, ca. 11x1,000nm, a possible member of closteroviruses[14,23]. Grapevine ajinashika disease is the most important viticulture virus disease in Japan[20]. Diseased trees show no visible symptoms and can be recognized only by a marked decline of sugar content in their fruits. Diseased fruits can neither be used as table grapes nor for wine. In this section we report the purification and some properties of GAV.

Fig. 1. Crystallines of GAV particles in phloem companion cell.

Fig. 2. Concentric membrane layers containing GAV particles in phloem parenchyma cell.

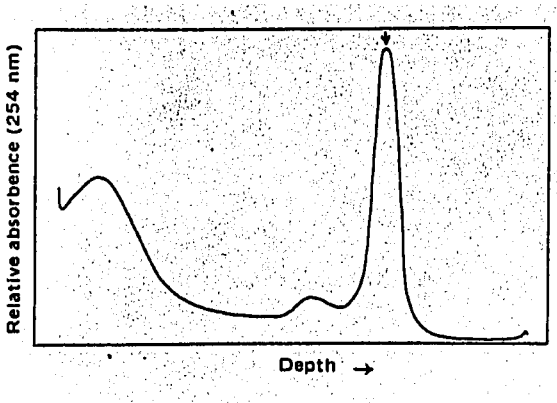


Fig. 3. Absorbance patterns of GAV, purified from infected grapevine tissue, after sucrose density gradient centrifugation.

Purification of GAV

Several purification schemes were tested for GAV. The enzyme extraction procedure using fruits resulted in the highest yields of virus and also gave cleanest preparations. Virus peak was obtained upon UV scanning of sucrose density gradients (Fig. 3).

Buoyant density of virions in CsCl

Isopycnic centrifugation in CsCl resulted in highly purified monodisperse preparations (Fig. 4). The buoyant density of GAV in $CsCl_2$ was about 1.38g/cm^3. The UV scan of purified preparations was typical of that of a virus.

Analytical ultracentrifugation

Purified preparations of GAV sedimented as single components with sedimentation coefficient of ca. 110s.

Electron microscopy

The virions of GAV are isometric with a diameter of 25nm in preparations negatively stained with PTA (phosphotungstic acid) (Fig. 5,6).

Nucleic acid

Unmodified nucleic acid of GAV gave single bands when electrophoresed on 2.5% polyacrylamide gels. The molecular weight was estimated to be ca. 2.3×10^6 (Fig. 7,8). Nucleic acid of GAV was susceptible to digestion by RNase, but not DNase.

Coat protein subunits

Electrophoresis of GAV coat protein in SDS-polyacrylamide gels revealed one major band and

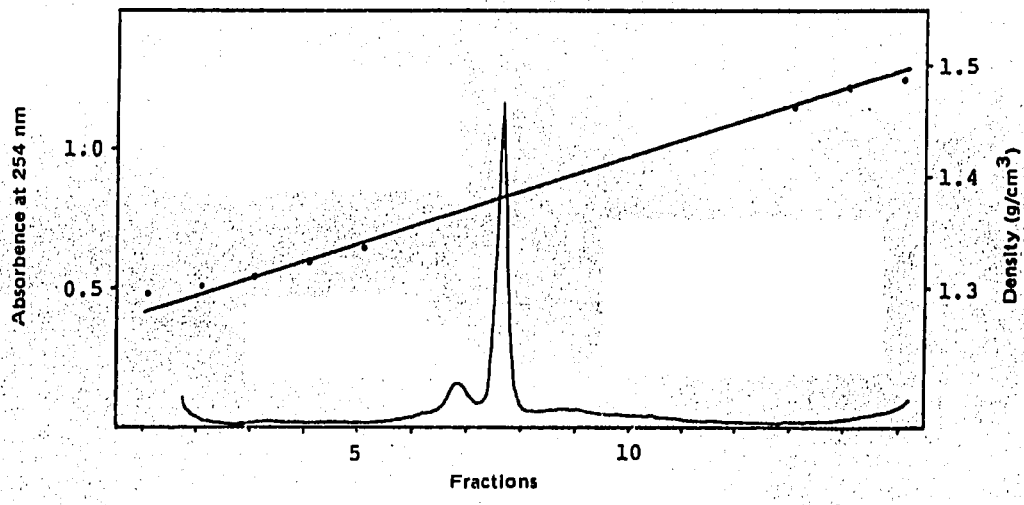


Fig. 4. Distribution of GAV after centrifugation for 30hr at 36,000rpm in $CsCl_2$ (ρ = 1.4 g/cm^3) in a Hitachi RPS40 rotor. The gradient column was analyzed using an ISCO UA-5 ultraviolet analyzer (smooth curve) and refractive indices (from which densities were calculated).

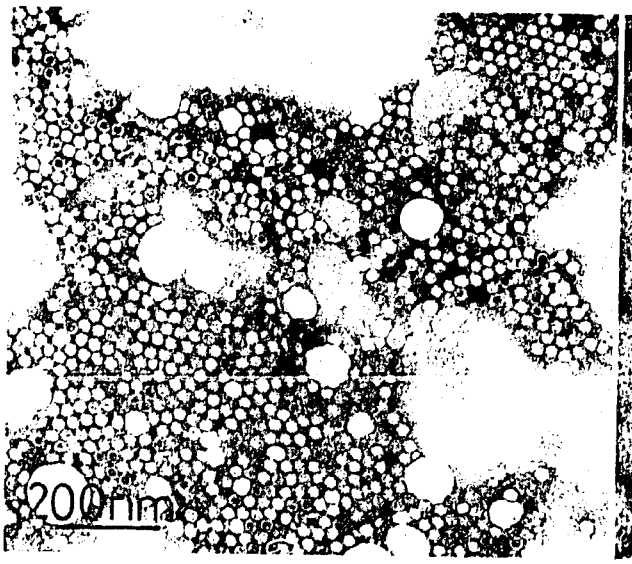


Fig. 5. Purified preparation of GAV.

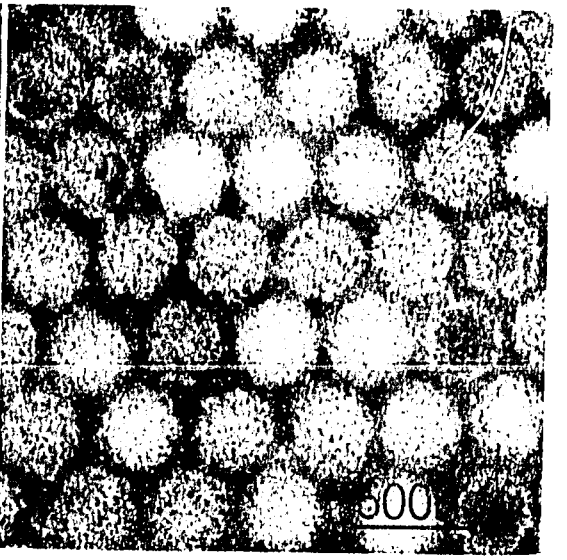


Fig. 6. High magnification of Fig. 5.

Fig. 7. Polyacrylamide gel electrophoretic analyses of RNA preparations. (a) 23S RNA (mol. wt. = 1.1×10^6) and 16S RNA (5.6×10^5) of *E. coli* ribosome and RNA (2.05×10^6) isolated from purified tobacco mosaic virus. (B) RNA isolated from purified GAV.

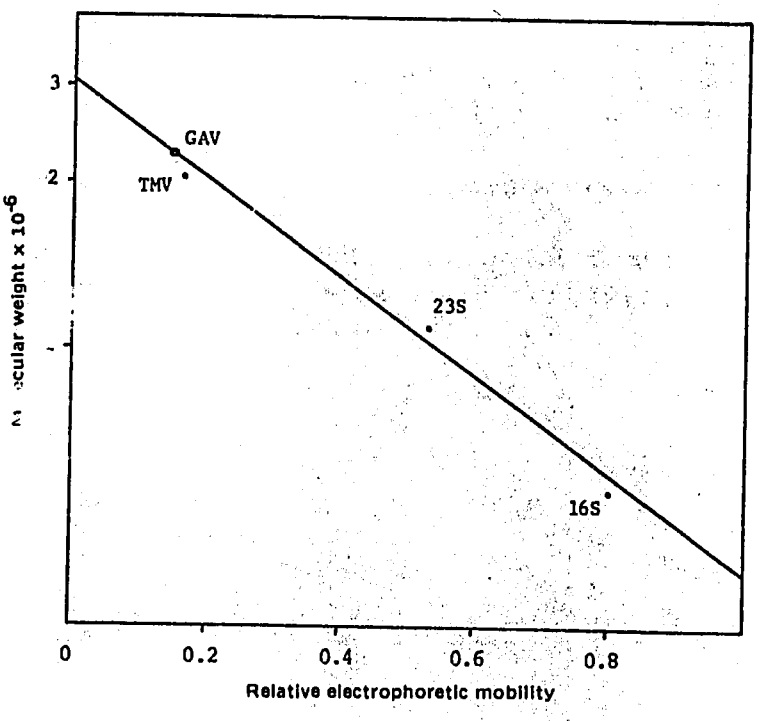


Fig. 8. Determination of molecular weight of grapevine ajinashika virus RNA by electrophoresis on 2.5% polyacrylamide gel. The line is obtained by plotting the molecular weights of marker RNAs against their relative mobilities. Abbreviations are: TMV, tobacco mosaic virus RNA (2.05×10^6); 23S RNA (1.1×10^6) and 16S RNA (5.6×10^5) of *E. coli* ribosome.

minor band (Fig. 9, 10). Using markers the molecular weight of GAV coat proteins were estimated to be 23,000 (major) and 24,000 (minor) respectively.

Detection of GAV by immunosorbent electron microscopy

In this procedure large numbers of virus particles were trapped and all of them were decorated with homologous antibodies (Fig. 11, 12).

Double diffusion tests

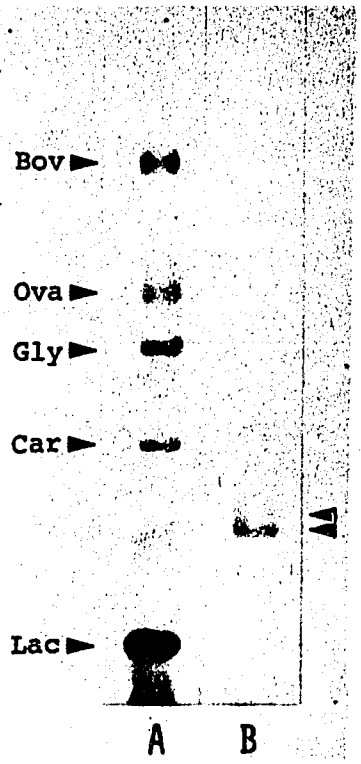
Antiserum to GAV had titers of 1/1,024 when tested against purified GAV in double diffusion tests in agarose gel (Fig. 13). In tests with partially purified GAV preparation from 125g of fruit, the GAV antiserum (diluted 1/16) reacted with it at dilutions up to 1/4 (Fig. 14). The tests using GAV antiserum and its homologous antigen failed to detect any serological relationships between GAV and the following viruses with small polyhedral particles: grapevine fanleaf virus (GFV), barley yellow dwarf virus (BYDV) and potato leafroll virus (PLRV) (Fig. 15, 16).

DISCUSSION

GAV was purified, sedimented as a single component of ca. 110S, had a buoyant density of ca. 1.4g/cm^3 ($CsCl_2$) and estimated nucleic acid content of ca. 30%[15]. GAV contained a single species of nucleic acid estimated mol. wt. ca. $2.3x10^{6}$[16], which suggested it was a single stranded RNA. Mol. wts. of GAV coat proteins were estimated to be 23,000 (major) and 24,000 (minor) respectively. GAV was detected from diseased fruit by immunosorbent electron microscopy[28]. With this technique, it is possible to detect diseased trees. Biological and physicochemical properties were similar to luteoviruses. But GAV had no serological relationships with BYDV and PLRV[16]. The following several small spherical viruses are isolated from grapevine: nepoviruses, tobacco necrosis virus (TNV), tomato bushy stunt virus (TBSV), broad bean wilt virus (BBWV), sowbane mosaic virus (SBMV), grapevine Joannes-Seyve virus (GJSV), Bratislava mosaic virus (BMV) and grapevine stunt virus (GSV)[2]. In symptoms and physicochemical properties, GAV is different from all of them except for GSV. Nepoviruses are of three types of isometric particle ca. 28nm in diameter with angular outlines, sedimenting at ca. 50, 90-120 and 120-130S and containing respectively ca. 0, 27-40 and 42-46% single-stranded RNA. Two RNA species, mol. wt. ca. 2.4x10 and 1.4-2.2x10 are both necessary for infection. Each particle contains a single polypeptide mol. wt. ca. 55,000. Virus particles occur systemically in tissues and are not phloem-restricted[8]. Antiserum of GFV, a member of nepoviruses, did not react with GAV in agar gel double diffusion test. TNV, TBSV, BBWV, SBMV, GJSV, and BMV are also different from GAV in morphological, physicochemical and biological properties (Table 1)[2,31]. GSV has some points of resemblance in particle morphlogy and location in plant tissues, but not in symptomatology. GSV causes marked stunting on vine but not decline of sugar content in fruits. GAV causes marked decline of sugar content but not stunting. So the two viruses differ. GSV was transmitted by grapevine leafhopper (*Arboridia apicialis* Nawa), it would be interesting to know the vector of GAV.

ELISA DETECTION OF GAV

Many growers attempted to control this disease by removing diseased trees. But it is difficult to replant healthy stock, because it is impossible to judge whether new nursery stock is healthy or not, until fruiting stage. Attempts were made to diagnose grapevine ajinashika disease by graft inoculation test with indicator test varieties of grapevine[29]. It has been suggested that fleck symptoms of St. George closely resemble the disease[28]; however, as more than a year is needed to diagnose by graft-inoculation test this is not yet confirmed. Recently GAV-antiserum was



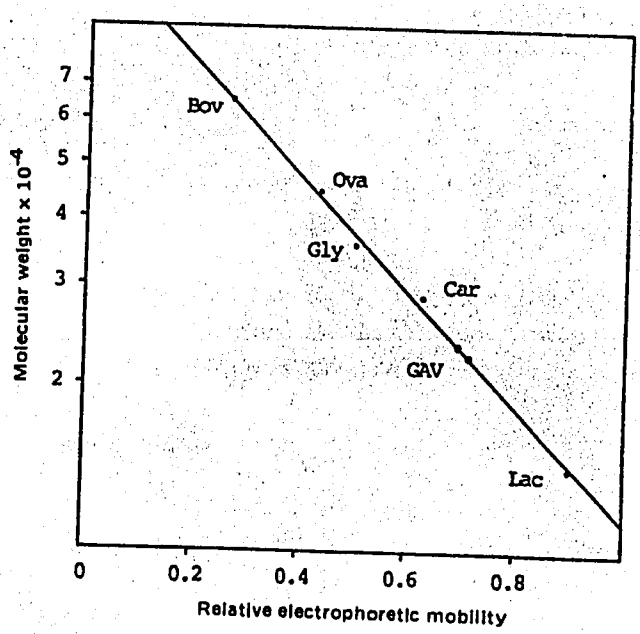


Fig. 10. Determination of molecular weight of grapevine ajinashika virus coat protein subunit by electrophoresis on 12% SDS-polyacrylamide gel. The line is obtained by plotting the molecular weights of marker proteins against their relative mobilities.

Fig. 9. Polyacrylamide gel electrophoretic analyses of protein preparations. (A) Marker proteins: Bov, bovine serum albumin (mol. wt. = 66,000); Ova, ovalbumin (45,000); Gly, glyceraldehyde-3-phosphate dehydrogenase (rabbit muscle) (36,000); Car, carbonic anhydrase (bovine erythrocytes) (29,000); Lac, α-lactalbumin (14,200) proteins as mol. wt. standards. (B) Dissociated GAV protein.

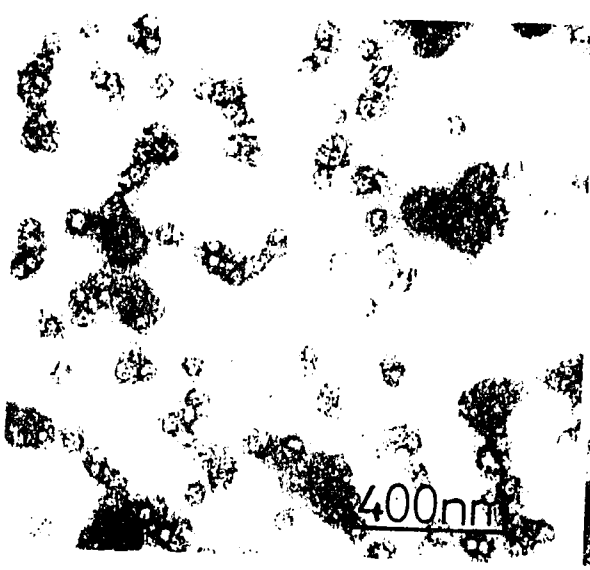


Fig. 11. Trapping decoration of GAV particles using GAV antiserum.

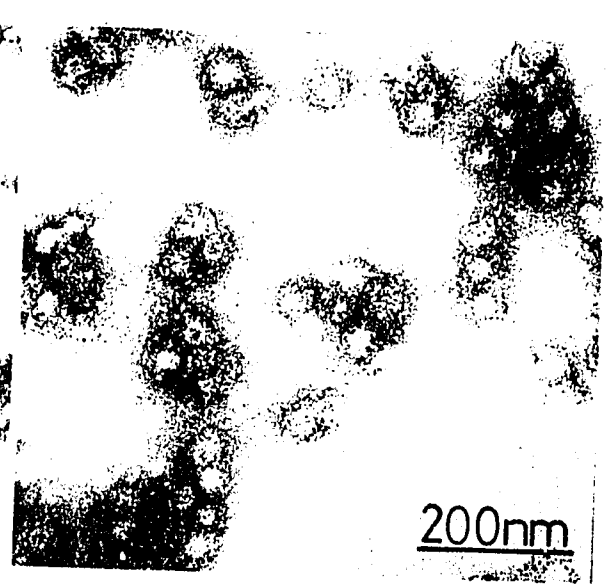


Fig. 12. High magnification of Fig. 11.

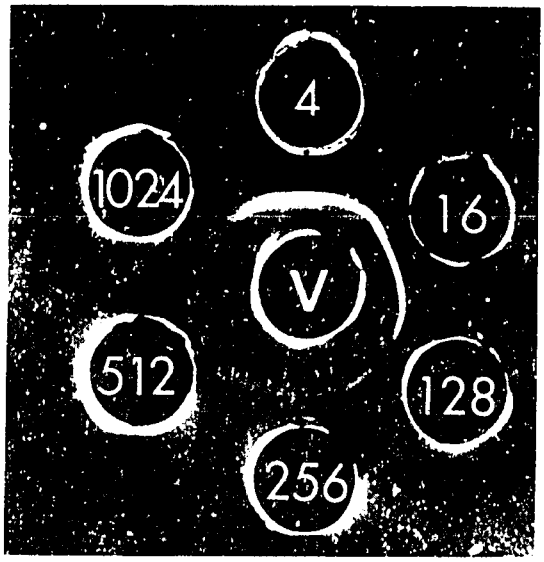


Fig. 13. Gel diffusion serology. Well (V): 1 μg of a purified preparation of GAV. The dilutions are indicated by a number: 4 means 1/4, 16 means 1/16, etc.

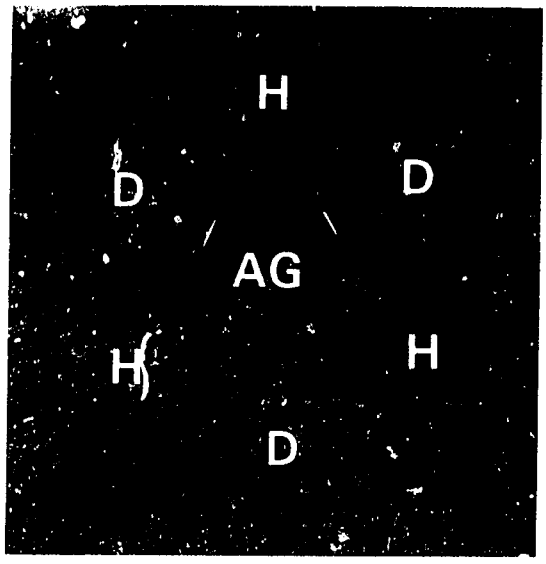


Fig. 14. Gel diffusion serology. Well (AG): GAV antiserum; well (D,H): partially purified preparations of GAV infected and healthy grapeving fruit core, respectively.

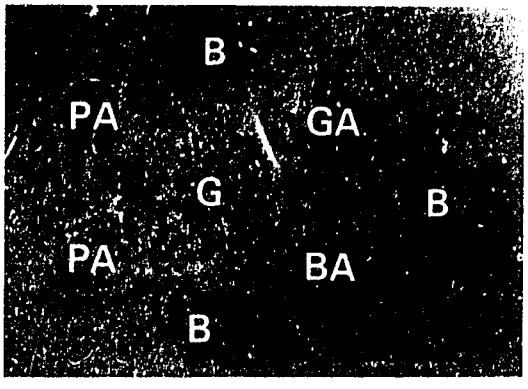


Fig. 15. Precipitation patterns of agar double diffusion reactions among two viruses (GAV (G) and BYDV (B)) and three antisera (GAV (GA), BYDV (BA) and PLRV (PA)).

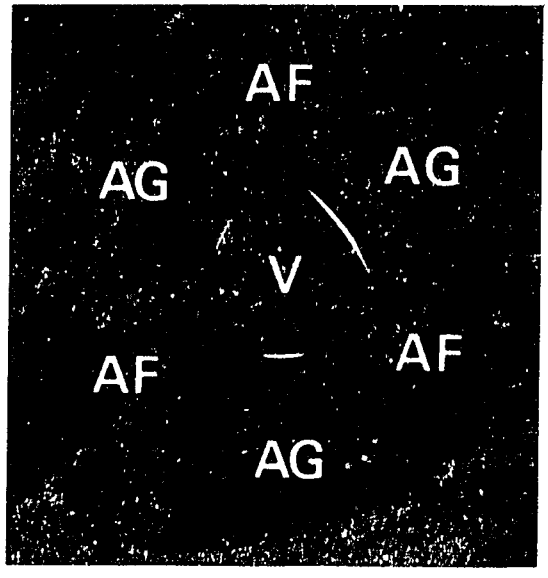


Fig. 16. Gel diffusion serology. Well (V): 1 μg of a purified preparation of GAV; well (AF): GFV antiserum; well (AG): GAV antiserum.

Table 1. Comparative properties of small spherical viruses detected from grapevine

	Particle size (nm)	RNA % of particle weight	Sedimentation coefficient ($S_{20,w}$)	Buoyant-density (g/cm3) ($CsCl_2$)	Mol. wt. of major polypetide (dalt.)	Mol. wt. of nucleic acid (x10^6 dalt.)	Location in plant tissues
GAV	25	30	110	1.38	23,000	2.3	Phloem-limited
GSV	25	—	—	—	—	—	Phloem-limited
TNV	25—30	18—21	112—133	1.399	30,000—33,000	1.5	Systemic
TBSV	30	17	140	1.350	41,000	1.5	Systemic
BBWV	25	33,22	63,100,126	1.40,1.44	42,000	20,1.53	Systemic
SBMV	26	20	104	1.43	19,200	1.3	Systemic
GJSV	26	—	—	—	—	—	Systemic
BMV	30	—	—	—	—	—	Systemic

prepared from purified preparation of GAV[16]. With agar gel double diffusion test, it is possible to detect GAV by extraction from fruit. However it would be more useful if it were possible to detect GAV in the young vine or dormant stick.

Enzyme-linked immunosorbent assay (ELISA) is a sensitive technique for the detection of plant viruses[4,30]. This procedure is capable of detecting low-titer and tissue-limited viruses, which would be difficult to assay by conventional means[1]. This paper reports the successful application of ELISA for detecting GAV[18].

Sensitivity of ELISA in detecting GAV

In preliminary test with purified GAV preparations, various ELISA absorbance values were observed with gamma-globulin dilutions of 1:100 (10μg/ml), 1:200, 1:400, 1:800, 1:1,600 and with conjugate dilutions of 1:100, 1:400 and 1:1,600. The sensitvity of coating gamma-globulin and conjugate to GAV in the experiments are shown in Fig. 17. The figure plots ELISA absorbance values obtained in test reactions between various concentrations of coating gamma-globulin and various concentrations of conjugate. For all further tests, coating gamma-globulin at 2.5μg/ml and conjugate at 1:400 were routinely used. Under these conditions, we tested the possibility of detecting GAV in purified virus preparations and extracts of leaf veins, petioles, young shoots, barks, young fruits and matured fruit cores. Purified GAV was detectable by ELISA at a concentation of 5ng/ml, and ELISA absorbance values gave a linear relationship to virus concentration over a range of values (Fig. 18). GAV could be detected in the experiments from 10^{-2} to 10^{-3} dilutions of matured fruit cores from infected grapevines (Fig. 19).

Detection of GAV in infected plants

A series of experiments was done to determine the reliability of ELISA for detecting GAV in infected plants. Many plant parts (leaf veins, petioles, current shoots, bark, young fruit and matured fruit cores) were sampled several times in each season. It was easiest to detect GAV from matured fruit cores (Fig. 19), and second easiest to detect from current shoots (Fig. 20). It was a little difficult to detect GAV from leaf veins and petioles. Young fruits were not suitable for ELISA to detect GAV. Almost all vines infected with grapevine ajinashika disease gave ELISA absorbance values high enough to diagnose and distinguish them from healthy vines, which gave

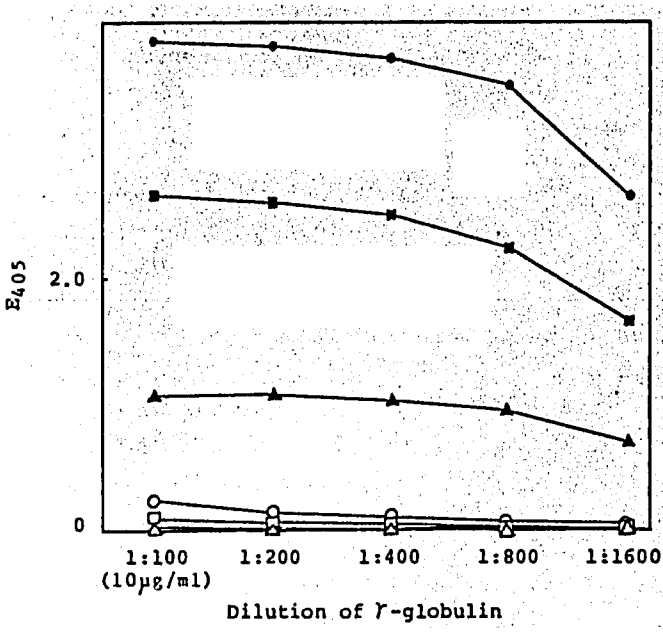


Fig. 17. Effect of concentration of coating γ-globulin and conjugate on ELISA absorbance values
Substrate solutions are diluted 1:4 in distilled water before measuring colorimetrically at 405nm.
Dilutions of conjugate: ●,○-1:100; ■,□-1:400; ▲,△-1:1600. ●,■,▲: purified GAV preparations (2.9 μg/ml); ○,□,△: healthy grapevine fruit core extracts.

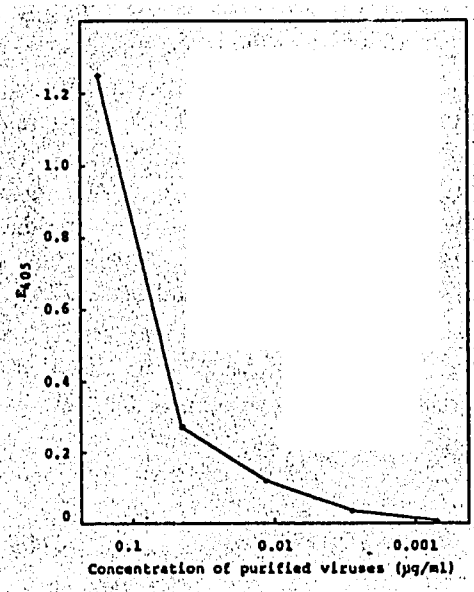


Fig. 18. Relationship between ELISA absorbance values and concentrations of purified GAV
Substrate solutions are diluted 1:4 in distilled water before measuring colorimetrically at 405nm.

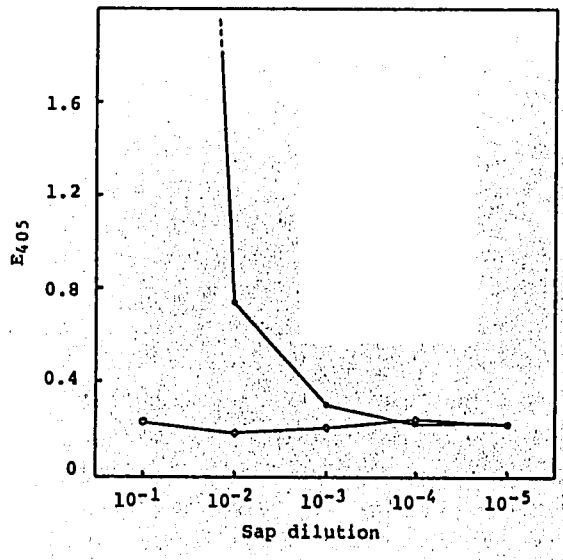


Fig. 19. ELISA absorbance values for GAV in diluted extracts of grapevine matured fruit cores infected (●) and healthy (○).
The precoating γ-globulin and conjugated globulin were used at 2.5 μg/ml and 1:400 dilution of the stock, respectively.

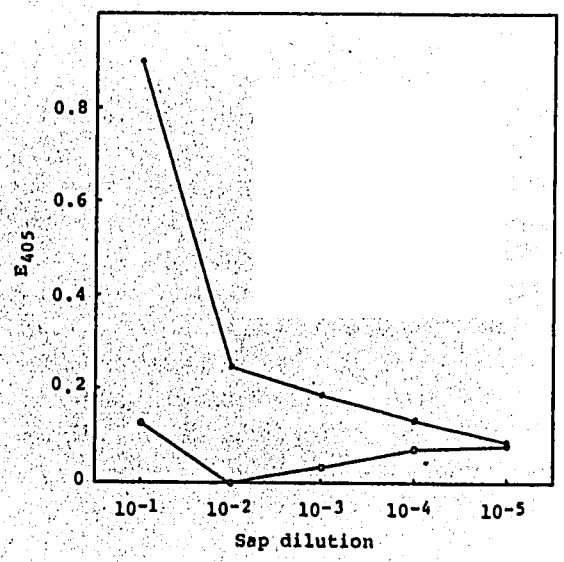


Fig. 20. ELISA absorbance values for GAV in diluted extracts of grapevine current shoots infected (●) and healthy (○).
The precoating δ-globulin were used at 2.5μg/ml and 1:400 dilution of the stock, respectively.

low ELISA absorbance values. Rootstocks were also tested for GAV. In Teleki 8B and Teleki 5BB, which have been used as rootstocks in Japan for a long time GAV was detected. Even Teleki 5BB, which was distributed as virus free rootstock, was revealed to be infected with GAV. Further GAV was detected from SO_4, which was also believed to be virus free. Two samples, Campbell Early infected with grapevine stunt virus and St. George showing fleck symptoms were tested. There were no clear differences in the ELISA absorbance values between these samples and healthy ones.

DISCUSSION

The ELISA test can be successfully used for detecting GAV in extracts of matured fruit cores, the extract of current shoots and purified preparations from grapevines. Extract dilutions of 1:10 were most effective to detect GAV. Matured fruit cores were most effective, but small young fruits were not good for detection. GAV is thought to be partly derived from grapevine rootstocks. In this study we tried to detect GAV from several rootstocks, among them Teleki 8B and Teleki 5BB which have been in long use, and Teleki 5BB and SO_4 which were necently distributed as virus free rootstocks. The former two proved to be infected with GAV; however, of the latter two, while GAV was not detected from Teleki 5BB, it was found to be in SO_4, supposed virus free. These results suggest two possibilities. The first is that GAV was not successfully eliminated from SO_4. The second is that SO_4, had been virus-free but became infected with GAV while planted in the nursery, which must be investigated further. ELISA absorbance values depend on plant parts in grapevine, and so, for diagnosis of GAV, low concentration and phloem-limited virus, we must take ELISA absorbance values for each plant part. The feasibility of using results from ELISA tests to control grapevine ajinashika disease depends on its suitability to test dormant wood for propagation. Niether Campbell Early budwood infected with GSV non St. George budwood showing fleck symptoms reacted with GAV antibody in ELISA. GSV has been assumed to be related to GAV. And fleck symptoms of St. George were suggested to be closely related to GAV[29]. For these results, there might be several reasons, perhaps virus concentration in tissues might be too low to detect by ELISA, or there might be a large enough antigenic difference to fail to give a positive reaction with ELISA. We are researching these relationships by sampling from various plant parts in grapevine at various stages and seasons.

GRAPEVINE STUNT VIRUS (GSV) DISEASE SYMPTOM AND TRANSMISSION

Stunt disease of Campbell Early grapevine (*V. vinifera* Linn.) has been observed in Okayama Prefecture for about ten years. At first known only in Okayama Prefecture, recently it was recognised in Oita, Fukuoka, Akita and Saitama Prefectures. Diseased grapevines tend to set smaller leaves which roll downward, and to develop dwarfed shoots. Recently, the disease was shown to be transmitted by the grapevine leafhopper, *Arboridia apicalis* Nawa, and to be caused by a small, spherical virus, which was named grapevine stunt virus (GSV)[9,21]. The grapevine leafhopper is widely spread all over Japan.

Symptoms

There are no symptoms upon sprouting, but later growth is remarkably reduced. In spring, development of shoots is remarkably inhibited, resulting in much shorter internodes. Diseased vines tend to set smaller leaves which roll downward, the tissue around the margins often being dried up and browned (Fig. 21). Later, severely affected leaves drop. The flower clusters on vines with stunt disease are usually smaller than those on healthy vines; they shell badly, and poor clusters are the rule. In advanced cases the vine fails to set fruit and the flower clusters wither away. Even if they set fruit, they set smaller fruit which may fail to seed. Symptoms of new shoots develop most markedly till about late in June,

from when on, newly expanded leaves show no visible symptoms. These symptoms show more prominently in young grapevines (1-4 years old).

Fig. 21. Leaf symptoms of grapevine infected with grapevine stunt disease.

Detection of causal agent

Small spherical virus particles were detected in negatively stained dip-preparations from diseased leaves of grapevine (Fig. 22). Since these particles were small in number, their detection was not easy. The particles were about 25nm in diameter.

Intracellular appearance of virus particles

The virus particles were also found in ultrathin sections prepared from both naturally and artificially infected plants of grapevine (Fig. 23). Their presence was always restricted to phloem parenchyma cells, companion cells and sieve elements. No virus particles were observed in other cells such as mesophyll cells and epidermal cells. In virus-infected cells the virus particles existed singly or as aggregates in the cytoplasm. They were not found in control prepartions from healthy plants of grapevine. In addition to the appearance of virus particles, virus-infected cells were characterized by the development of small vesicles containing fibrous materials in the cytoplasm or vacuole. The virus particles were always associated with these vesicles. However, the vesicles were not always accompanied by the virus particles. The small vesicles, about 60-100nm in diameter, were surrounded by a single-layered membrane, containing fibrous materials resembling nucleic acids so far reported in plant cells infected with some

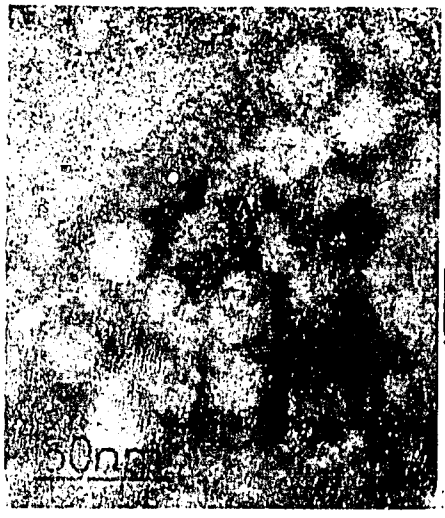


Fig. 22. Negatively stained GSV particles

Fig. 23. Phloem necrosis and GSV particles

viruses. Virus particles were often observed in plasmodesmatas connecting to neighboring cells. Phloem necrosis was usually observed in diseased plants. It seemed to cause the accumulation of starch grains in chloroplasts in the mesophyll cells.

Transmission of the virus.

(1) *Mechanical transmission* Attempts to transmit the virus to healthy seedlings of grapevine and several indicator plants by inoculation of sap were unsuccessful.

(2) *Transmission in soil* Healthy grapevines (Campbell Early) and diseased ones were planted respectively in unglazed pots or concrete boxes filled with soils from diseased orchards. Within a year, stunt symptoms were observed on originally diseased grapevines, but no symptoms were observed on originally healthy grapevines.

(3) *Graft transmission* Clones of diseased and healthy Campbell Early vines were selected in Okayama, 1979. Diseased clones showed some variation in symptoms that ranged from mild to severe stunting. Healthy test plants were taken from the Foundation Plantings, Okayama Prefectural Agricultural Experiment Station, Okayama. In 1980 and 1981, healthy root cuttings, nursery stocks and mature trees were inoculated by chip-bud grafting with several clones of the diseased Campbell Early. Readings for symptoms were made in 1980 and 1981. The plants were examined for stunting. In less than a year rooted cuttings and nursery stocks inoculated with diseased clones showed stunting symptoms (Table 2,3) while mature trees showed symptoms about a year later (Table 4). All of tested plants inoculated with healthy clones showed no symptoms.

Table 2. Graft inoculation test by chip-bud grafting to rooted cuttings

Inoculum	No. of trees infected/tested	
	Exp. 1 (1980)	Exp. 2 (1981)
Diseased*1	17/26	23/56
Healthy*2	0/22	0/25

*1. Clones of diseased Campbell Early
*2. Clones of healthy Campbell Early

Table 3. Graft inoculation test by chip-bud grafting to nursery stocks

Inoculum	No. of trees infected/tested
Diseased*1	4/11
Healthy*2	0/5

*1. Clones of diseased Campbell Early
*2. Clones of healthy Campbell Early

Table 4. Graft inoculation test by chip-bud grafting to mature trees

Inoculum	No. of trees infected/tested
Diseased*1	5/12
Healthy*2	0/3

*1. Clones of diseased Campbell Early
*2. Clones of healthy Campbell Early

(4) *Transmission by grapevine leafhopper* The virus was easily transmitted to healthy grapevine (Campbell Early) by viruliferous insects of the grapevine leafhopper (Fig. 24). Acquisition feeding of five days followed by inoculation feeding of seven days is necessary for infection. The first symptoms of setting smaller leaves which roll downward appeared on the inoculated vine about a year after inoculation, and later various symptoms appeared on shoots and clusters. These symptoms were all the same as those observed in naturally infected plants of commercial orchards. In addition, the virus particles were also detected in ultrathin sections from these artificially infected plants. The details of virus vector relationships are now being studied. Both these symptoms and cytological changes were also observed on diseased grapevine with graft inoculation.

DISCUSSION

Grapevine stunt disease occurs almost everywhere Campbell Early grapevine is planted. GSV was transmitted by grafting and by grapevine

Fig. 24. Grapevine leafhopper (*Arboridia apicalis* Nawa).

leafhopper (transmissibility was about 50%)[10,17]. Cicadellidae is a new insect vector of plant virus. GSV is small spherical and phloem-limited virus. It's properties are similar to GAV. But GSV reacted little with GAV in ELISA test[18]. We are now trying to make GSV-antiserum to detect the relationship between the two viruses. GSV was also detected from Ebizuru (*Vitis thunbergii*) by electron microscopy and tested with grapevine leafhopper. Ebizuru grows wild in Japan and may be one of the sources of infection. GSV was diagnosed by direct fluorescence detection (DFD) method[22].

GRAPEVINE LEAFROLL VIRUS

Leafroll of grapevine occurs widely in the world and is an important problem. Foliar symptoms of the disease are usually evident after early autumn. The leaves roll downward and turn red (or yellow) progressively. Diseased vines have smaller clusters, fewer clusters per vine, and the sugar content is decreased, although the decrease is not so remarkable as that in case of the grapevine ajinashika disease[19,20]. The leafroll is transmitted by grafting, and considered to be a virus disease. The causal virus, however, has not been detected in diseased vines under the electron microscope. Tanne *et al.* (1977)[27] reported that the grapevine leafroll virus was a potyvirus, but this has not been confirmed by other researchers. In 1979, we reported that the grapevine leafroll virus (GLRV) was a long, flexuous, rodshaped virus, which existed only in the phloem tissue of diseased vines[23].

In Japan, the disease was first recognized in 1966[26], and recently the disease was shown to be widespread in most grapevine cultivars. Terai and Yano (1979) detected the leafroll in fourteen grapevine cultivars by indexing tests on LN-33 vines[28]. In our study a number of vines were examined for the presence of the GLRV under the electron microscope. Leaves and fruits from diseased vines of nine cultivars (Koshu, Kaiji, Lungen, Gros Semillon, Cabernet Sauvignon, Cabernet Franc, Flame Tokai, Campbell Early and Kyoho) showing the typical leafroll symptoms were collected in Yamanashi, Nagano and Okayama prefectures in 1976, 1977, 1978 and 1979. In the same years, leaves and fruits from diseased vines of three cultivars (Koshu, Cabernet Sauvignon and Pinot noir), which had been proved to be infected with the leafroll by indexing tests on LN-33 vines, were collected at the Yamanashi Fruit Tree Experiment Station. As controls, leaves and fruits from healthy vines of six cultivars (Koshu, Pione, Delaware, St. George, Campbell Early and Kyoho) showing no leafroll symptoms, were collected in Yamanashi, Nagano and Okayama Prefecture in 1976, 1977, 1978 and 1979. In the same years, leaves and fruits from healthy vines of two cultivars (Fuefuki and Pinot noir), proven free of the leafroll by the indexing tests, were collected in Yamanashi Fruit Tree Experiment Station. All these leaves and fruits were used as materials in our study. In 1977, diseased leaves from Pinot noir vines infected with the leafroll and healthy ones were collected from the same cultivar in California, U.S.A., prefixed in 5% glutaraldehyde, and brought to Japan for our

Fig. 25. GLRV particle in negatively stained dip preparation.

Fig. 26. Phloem cells showing necrosis, and aggregated masses of GLRV particles in sieve tube.

study. Long, flexuous, rod-shaped particles, approximately 11x1,000nm, were consistently observed (Fig. 25) in negatively stained dip-preparations from diseased leaf materials and in the prefixed preparations of diseased leaves from California, but not in healthy control preparations. Leaves, peduncles and fruit cores from diseased vines were fixed in 5% glutaraldehyde, postfixed in 1% osmium tetroxide, embedded in Epon 812, thin sections cut, stained with uranyl acetate and lead citrate, and examined under an electron microscope. Controls were prepared from healthy vines in the same way. In all thin sections from diseased vines, phloem necrosis and accumulation of starch grains in mesophyll cells, and long, flexuous, rod-shaped particles, approximately 9x 1,000nm, in all the diseased materials and never in healthy controls.

These results strongly suggest that GLRV is a long, flexuous, rod-shaped particle which is restricted to phloem cells and transmitted by grafting. This, along with the intracellular appearance of virus-infected cells, suggests that GLRV may be a member of closteroviruses. GLRV is not transmitted by inoculation of sap[7]. Transmission by mealybugs was suggested, but

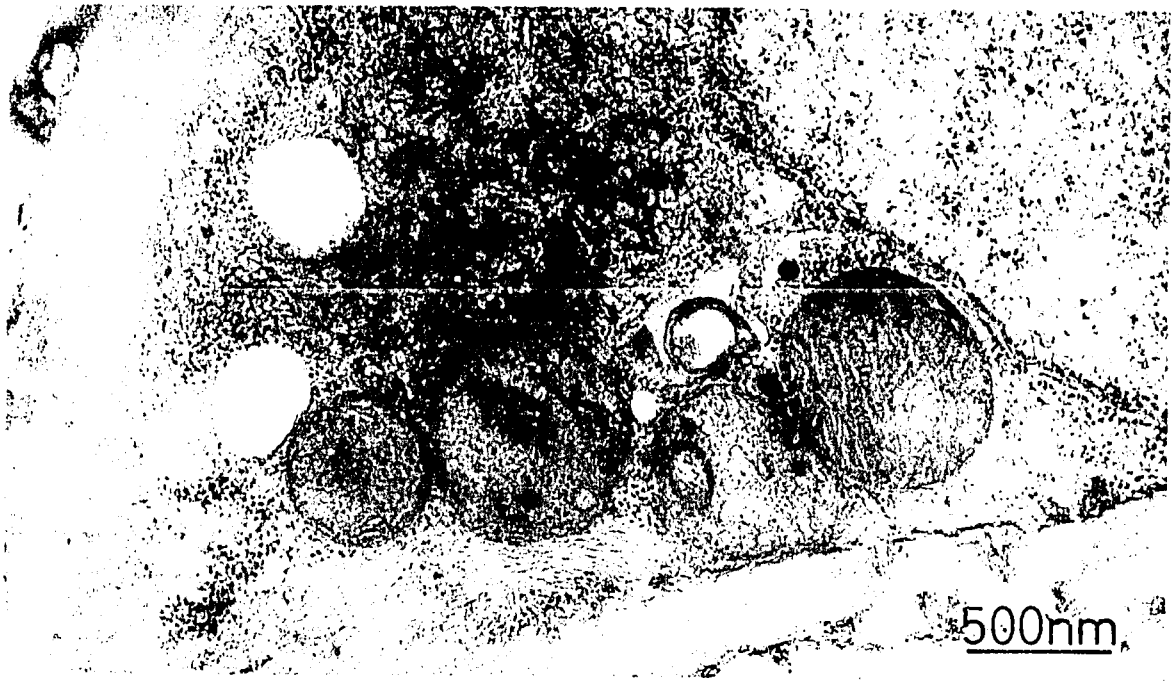


Fig. 27. Development of vesicular structures associated with GLRV particles in phloem companion cell.

there is no conclusive evidence[25].

Tanne *et al.* (1977) detected a potyvirus in *Nicotiana glutinosa* plants mechanically inoculated with phenol extract from leafroll-infected grapevine leaves[27], but they did not succeed in detecting this virus in diseased vines. In Japan, two potyviruses were isolated from grapevines to herbaceous test plants. One was isolated from grapevine showing mosaic leaf symptoms[11], and another was from that showing ring pattern mosaic symptoms on fruit[12]. Both were also detected in phloem cells, and further, sometimes particles were observed passing through the plasmodesmata, which were thought to correspond to those detected in dip preparations. The particles sometimes existed as aggregated masses or crystallines in the cytoplasm of sieve tubes, phloem companion cells or phloem parenchyma cells, which usually collapsed to show the phloem necrosis (Fig. 26). The distance from center to center of two neighboring particles in a crystalline array was about 9nm. Characteristic vesicular structures containing fine fibrils were often observed in phloem cells (Fig. 27). The particles were also found in diseased leaves from LN-33 indicator vines infected with the leafroll in indexing tests, but not in healthy ones. In summary, the particles were detected in grapevine tissues.

Recently studies have been done on closterovirus-like particles and high molecular weight double-stranded RNA found in leafroll-infected grapevine cultivars[3,14]. Closterovirus-like particles were observed in phloem tissues of leafroll-infected vines, but not in those of virus-free ones. Particles had a modal length of about 1,400nm in negatively-stained preparations. Double-stranded RNA, which has a mol. wt. of about 8x 10^6, was isolated from diseased grapevines, but not from healthy ones. In a further study of grapevine virus A (GAV) and grapevine virus B(GVB)[13]; GVA was shown to be a closterovirus, diameter 11-12nm, pitch of 3.6-4.0nm and a modal length near 800nm[5], closely related to grapevine stem-pitting disease[5] and transmitted by Pseudococcidae mealybug (*Pseudococcus longispinus*)[25]; and GVB a closterovirus type, of the same diameter and modal length unknown but probably

larger than GVA. GVB is closely related to grapevine leafroll disease[13].

These findings suggest that grapevine leafroll virus occurs widely in the world. Further studies should clarify whether these long flexuous, rod-shaped viruses are identical or not.

DIAGNOSING GAV, GSV AND GLRV BY DFD

The fluorescence microscope is widely used not only in biology but also in medicine, chemistry, biochemistry, pharmacology and industry. In plant virology the fluorescent antibody technique (immunofluorescence technique) and staining methods with fluorescent dye, developed in medicine, are used to observe plant tissues infected with viruses[6] and MLOs[24]. However, to date, the transmitted light fluorescence microscope technique by the conventional tissue preparation embedding, cutting with microtome and staining is a tedious procedure. Direct Flourescence Detection (DFD) method is a simplified method for diagnosing various yellows-type virus diseases and mycoplasma diseases caused by phloem-limited agents (viruses and MLOs)[22]. When observed under a reflecting fluorescence microscope (RFM), fluorescent cells distinctly showed a yellow fluorescence in their phloem tissues, never seen in cells of healthy plants. By electron microscopy of the same materials, the fluorescence was proved to originate in necrotic phloem cells.

Practical DFD method

(1) *Sample materials used for diagnosis.* Leaf vein, petiole flower peduncle, fruit peduncle, core and current stem are all useful for diagnosis. Since dormant branches can also be used, it is applicable for diagnosis in nursery stocks.

(2) *Preservation of samples.* Frozen (−20°C to −70°C) as well as refrigerated samples can be used for diagnosis. Frozen samples can be stored at −20°C for up to several months and at −70°C for up to several years. Tissues can be fixed with 100% ethanol or 2% glutaraldehyde solution (in 0.1M phosphate buffer pH7.0). In 100% ethanol samples can be stored almost indefinitely and also investigators can exchange samples by shipping in a fixing solution.

(3) *Preparation of samples.* Cutting thin sections with microtome, fixing or staining is unnecessary. The sample mounted on a slide glass, can be of any practical thickness (Fig. 28). First, a tissue sample which contains a vascular bundle such as leaf vein, petiole, shoot, stem, root, flower and fruit etc. is cut and mounted on a slide glass with water, and then a non-fluorescent cover glass is placed over it. Any razor blade may be useful to cut sections. The slide can be frozen for preservation.

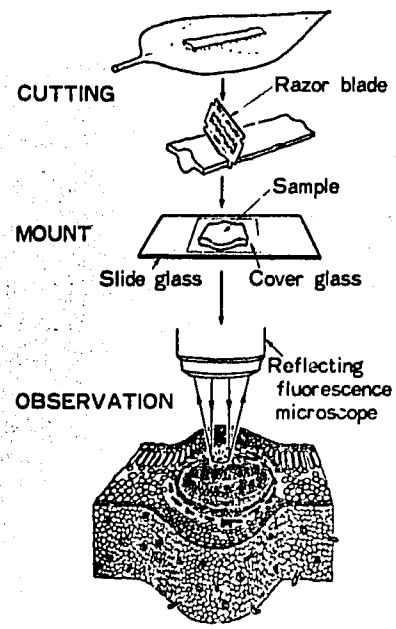


Fig. 28. Procedure of DFD method.

(4) *Diagnostic method.* When observed under RFM with a blue excitation light, samples distinctly show yellow to green autofluorescence in the xylem and green in the phloem fibre. Samples of infected plants show yellow to green color fluorescence of shrunken cell groups in phloem tissue, while such fluorescent cells are never found in healthy tissue. By electron microscopy of the same samples, causal agents (virus or

MLO) were observed in phloem cells and adjacent to phloem cells, which were fluorescent under RFM.

DISCUSSION

Using DFD method, it is possible to diagnose virus or MLO infection of plants in its early stages even in dormant or early growth stages, enableing early detection of diseases. It is especially useful for diagnosis in plants such as fruit trees or potatoes for vegetative propagation. This method can expedite diagnosis of GAV, GSV or SLRV.

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INDEXING AND CONTROL OF CITRUS VIRUSES AND VIRUS-LIKE DISEASES

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ABSTRACT

Concepts for the control of citrus virus diseases include quarantine and eradication programs, use of nucellar lines and more recently thermotherapy and shoot tip grafting as a better means of obtaining virus-free citrus stock. A citrus industry today must be based on indexed virus-free mother or foundation stock which is extended to a budwood certification program.

Recent developments in laboratory testing of citrus include culturing Spiroplasma citri for detection of stubborn disease, the development of ELISA and other serological techniques for the detection of tristeza and other viruses and the detection of ds RNA by electrophoresis. However, viruses can be missed by the ELISA test and therefore plant indexing remains the means of critical evaluation for certification.

摘 要

防治柑桔病毒之原理包括利用檢疫與撲滅計畫，以及珠心胚、熱處理法與莖頂嫁接等方法，可以獲得無病毒柑桔之材料。今日之柑桔產業，必須以無病毒之種源爲基礎，再發展爲苗木檢定計畫。

目前柑桔試驗檢定之發展，包括*Spiroplasma citri*的培養以檢定較難防治之病害，ELISA與其他血清方法檢定南美柑桔立枯病與其他病毒以及利用電泳法檢定ds RNA。ELISA有時無法檢驗出病毒，因此仍需保留指標植物做爲檢定之主要方法。

摘 要

檢疫や根絶計畫を含めた柑橘のウイルス病防除對策において、珠心胚系の利用、最近では熱療法や新芽先端接木法は無病株を得る良い方法である。今日の柑橘産業では芽の檢定計畫までを含めた檢定濟の無病母樹を使用する必要がある。柑橘の室内檢定法の最近の進步として、stubborn病檢出の爲の*Spiroplasma citri*の培養、ELISA その他の血清學的諸方法による Tristeza virus その他のウイルスの檢出、電氣泳動による二本鎖 RNA の檢出法がある。

しかし、場合によつてはELISAでウイルスを檢出できないことがあるので、植物を使つた檢定も必要である。

INDEXING AND CONTROL OF CITRUS VIRUSES AND VIRUS-LIKE DISEASES

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The history of citrus production throughout the world records many dramatic upheavals and changes directly attributed to diseases. The disasterous *Phytophthora* epidemics of the mid 19th century were responsible for the worldwide destruction of citrus seedling trees[10], and transformed the industry based on seedlings to one based on *Phytophthora*-tolerant or resistant rootstocks. The sour orange stock, first used in Spain, was adapted successfully in those areas of the world where tristeza (then unknown) was nonendemic. However, sour orange was found to be incompatible with sweet orange, grapefruit or mandarin scions in many parts of Asia, South Africa, Australia etc., and budded trees would die within a few years after planting. Only after the discovery of the Mexican or Key lime as an indicator for tristeza[33] did it become evident that the tristeza virus was responsible for this incompatibility. However, in some areas of the world, specifically in Argentina and Brazil, where vast plantings of citrus were predominantly or sour orange stock, epidemics occurred, and millions of trees were destroyed. The presence of an efficient vector for the virus (*Toxoptera citricida*) plus the presence of the more virulent seedling yellows strains of this virus complex created a disaster unparalleled in the history of fruit-tree production[2,3]. The disease also developed in southern California, Florida, Spain, Israel and more recently in Venezuela where many millions of trees on sour orange stock were destroyed and are still being destroyed. Once tristeza is established in an area, there is a continual search for new rootstocks to replace the sour orange. Unfortunately, randomly selected propagative budwood was later found to contain other 'viruses'* which caused other problems. Exocortis is destructive to Rangpur lime, trifoliata, sweet limes and many citrange stocks; cachexia is destructive to Cleopatra mandarin, sweet limes, *Citrus macrophylla*, and tangelos; tatterleaf is destructive to trifoliata and many citranges. Even when tristeza is well established, new strains may appear or be imported; they may superimpose on existing strains and new declines can occur.

Another equally serious citrus disease is currently destroying millions of trees in Asia, Africa and areas of the Pacific. This is the greening disease with its psyllid vectors which must be considered as one of the most serious and potentially destructive disease problems of citrus. It presents critical problems wherever present and even where it is not present, its threat is ominous, for it is moving and it can adapt to new areas and new situations[5].

The rapid spread of a psorosis disease in Argentina suggests that a normally non-vector transmissible virus can change its character and become highly transmissible. This disease now is a serious threat in this region and presents a good argument for the elimination of trees with psorosis

*The general term 'viruses' will be used to include the viroids, mycoplasmas, *Spiroplasma citri*, greening organisms, and other virus-like pathogens.

lesions in countries where psorosis-A is present but not spreading naturally.

The mechanically transmissible 'viruses' such as exocortis, cachexia, tatterleaf, infectious variegation, satsuma dwarf (and its related viruses) can disseminate rapidly within an area by normal pruning and fruit picking practices. These pathogens must be considered as serious pests and should not be allowed to be distributed inadvertantly in newly released budwood.

Today, techniques are available for the relatively rapid detection of most citrus 'viruses'. Also, techniques are now available for the effective elimination of perhaps all citrus 'virus' diseases

THE MAJOR CITURS VIRUS DISEASES – WORLDWIDE

Citrus virus diseases may be classified as follows:

Diseases	*Organisms*
A) Primarily vector transmitted	
1. Tristeza Complex	Closterovirus
– tristeza sweet/sour decline	
– seedling yellows tristeza	
– stem pitting tristeza	
2. Greening disease	Gracillicutes bacteria
– Asian	
– South African	
3. Stubborn disease	*Spiroplasma citri*
4. Vein enation	probable virus
B) Primarily mechanically transmitted (and also bud transmitted)	
5. Exocortis	Viroid
6. Cachexia (Xyloporosis)	probable viroid
7. Infectious variegation	ilarviruses
– satsuma dwarf	(26-33 nm)
– navel infectious mottle	
– natsudaidai dwarf	
8. Tatterleaf	virus
C) Primarily bud-graft transmitted (by man)	
9. Psorosis-A	probable virus
ringspot	virus
10. Concave gum (oak-leaf patterns)	probable virus
impietratura	
cristacortis	
D) Serious diseases of unknown etiology which are spreading	
11. blight	unknown
12. fruita bolita (misiones disease)	unknown
13. Argentine psorosis	unknown

A more detailed and illustrated description of these diseases can be found in Bové and Vogel's excellent slide collection and text[4] or in Wallace[34].

from propagative budwood. This paper will review virus and virus-like diseases of citrus from the viewpoint of their detection and elimination from propagative budwood.

CONCEPTS FOR CONTROL OF CITRUS VIRUS DISEASES

Quarantine and eradication programs

The importance of strengthening quarantine laws cannot be overemphasized. Nearly all citrus diseases have been transported from one area or country to another inadvertently without knowledge of their potential pathology[2,3]. Today there should be no excuse for disseminating diseases by individuals bringing in infected trees, soil or propagative material. Concepts and procedures have been developed for importing citrus budwood[30]. The potential dangers from illegally introduced pathogens and pests are immeasurable. One can well imagine the consequences and problems which would follow if the Asian greening disease and its vector *Diaphorina citri* were introduced into a new area where the disease is not present and climatic conditions are suitable for the vector. It could well result in the destruction of the citrus industry of that region.

Use of nucellar lines for virus free citrus

With the recent advent of thermotherapy and shoot tip grafting for the elimination of pathogens from old clone citrus budwood, and the uniform true-to-type progeny obtained by shoot tip grafting[21] the need for nucellars as a means of bypassing 'viruses' may now be outdated. There may be very few advantages in developing nucellar budlines today. Nucellar lines take from four to eight years to come into fruit and many lines do not bear fruit. The fruit quality of nucellar lines, with some exceptions, may be poorer than the old lines from which they were derived. Such characters as looseness of core, coarser flesh texture, thicker, coarser, puffier and pebbled rinds, tapered and furrowed stem ends and larger columellas may take years to disappear, and some of these characters do not completely disappear. Other undesirable juvenile characteristics associated with nucellars are: excessive thornines, upright growth habit, excessive tree vigor, alternate bearing, late flowering and fruiting, unequal fruit distribution and a more rapid deterioration in quality of fruit on the tree, especially with navel oranges.

Thermotherapy

A number of techniques are available for heat-treating budwood. These have been reviewed in detail by Roistacher[29], and will be only briefly summarized here. Preconditioning of plants prior to their use, is vital to success[25]. Plants should be grown (preconditioned) at warm temperatures (32-40°C day and 26-30°C night) for one to three months prior to budwood treatment. A preferred method of heat treatment[29] (Fig. 1C), involves treatment of buds grafted on tolerant stocks in a lighted chamber. Buds cut from preconditioned plants are grafted to heat-tolerant rootstocks such as Troyer citrange or Rangpur lime[7]. The budded plants are placed in a chamber at high humidity and at an initial temperature of 38/30°C (38°C for 16 hours with lights and 8 hours at 30°C in the dark). After one week the temperature is raised to 40/30°C. The budded plants are held at this temperature regime for 8 to 12 weeks, after which they are removed to the greenhouse and the buds forced. New growth is then indexed to verify virus inactivation. Another thermotherapy method is the treatment of preconditioned budsticks in an incubator. Budsticks 10-12 cm in length are placed in a plastic container with a small quantity of water at the base and held in an incubator for 3 to 22 hours at 50°C (29, Fig. 1B). Other techniques for thermotherapy may be used and are described and illustrated elsewhere[7,29]. Thermotherapy has successfully eliminated the viruses of the tristeza complex including seedling yellows and stem pitting, psorosis-A and B, concave gum, impietratura, infectious variegation, vein enation, tatterleaf and Asian and South

African greening. Thermotherapy has not eliminated the exocortis, cachexia, yellow vein or stubborn pathogens from citrus budwood.

Shoot Tip Grafting

The grafting of a very minute shoot tip consisting of the meristem and 2 to 4 leaf primordia measuring 0.14 to 0.2 mm has successfully eliminated nearly all of the known citrus viruses[15,22,24,28,31]. This is especially important for the exocortis and cachexia pathogens which are extremely heat tolerant and are very difficult to eliminate by thermotherapy. Plants derived from shoot tip grafts cannot be assumed free of 'viruses' and must be indexed to be sure 'viruses' are not present. This holds equally true for heat-treated plants. A combined procedure using thermotherapy and shoot tip grafting should assure the elimination of perhaps all known citrus 'viruses'. However, intensive and complete indexing is an absolute prerequisite.

Indexing

Fundamental to any program for the control of citrus 'viruses' is a sound indexing program and facility. Dispite dramatic advances in biochemical and immuno-assay technology, the plant still remains as the eyes through which we can see and identify most citrus 'viruses' (Table 1). Therefore, any program for the eradication of citrus 'viruses' must include a plant laboratory with facilities for producing the finest plant growth[20,27]. It is important that indicator plants develop vigorous clear leaf flushes free of micronutrient deficiencies or excesses, and be maintained as free as possible of insect pests. Temperature control is important since good virus detection is temperature dependent. Certain viruses i.e., psorosis, tristeza, concave gum, infectious variegation, vein enation etc., are best seen in plants grown under cool conditions while other pathogens i.e., exocortis, cachexia, stubborn etc., are best seen under warm conditions. An ideal facility must therefore have provisions for a cool room or a section held at 26-30°/18-20°C, a growing section at 28-30°/20-22°C, and a warm section at 32-40°/24-30°C. (Maximum day temperatures/minimum night temperatures.)

A good growing media and a balanced supply of plant nutrients are the heart of good plant production. At the University of California at Riverside we have studied and modified the UC mix for production of good citrus plants[17,19,20]. It consists of mixing equal parts of fine sand, Canadian peat moss and wood shavings (equal parts of fine sand and peat moss are acceptable). To this inert mixture, the macronutrient salts of calcium, magnesium and phosphorous plus the micronutrient salts of copper, zinc, iron, managanese, molybdenum and boron are added. A pH of 5.0 to 6.5 is maintained by balancing ammonium and calcium nitrate in the liquid feed. Potassium is also supplied in the liquid feed as muriate of potash. The importance of developing a good plant laboratory for detection of citrus viruses cannot be overemphasized.

Recent Developments for Laboratory Testing of Citrus 'Viruses'

There is a valid need to replace the plant as an indicator for citrus 'viruses' because of the time requirements, difficulty and expense in maintaining a good plant laboratory. There has been a continual search for alternatives and progress is being made. Two areas of success are noteworthy. The culturing of *Spiroplasma citri* for detection of stubborn disease[11,32] and the development of ELISA (enzyme-linked immunosorbant assay) and other antigen-antibody techniques for the detection of tristeza[1,12]. This has permitted large-scale indexing for tristeza in areas where the virus has not become endemic, such as in the central valley of California, Israel and in southern Spain. In Japan, the ELISA technique has been used in a nationwide campaign to certify satsuma mandarins free of citrus mosaic virus[16]. If and when citrus viruses can be purified and antiserum produced, the ELISA and similar immuno-assay techniques will be most helpful and desirable to have as

Table 1. Major citrus virus and virus-like diseases and their detection

Disease	Plant Index (by bud transmission)[1]	Other Indexes
Blight	none	– Water uptake – Zinc accumulation in bark – Amorphous plugs in xylem – Phenolic content in bark
Cachexia (xyloporsis)	– Parson special mand. forced on a vigorous rootstock (i.e., ro. le.); – Orlando tangelo sdlgs.	
Concave gum	– Dweet, mand. or sw. o. sdlgs. for oak leaf pattersn	
Cristacortis	– Orlando tangelo sdlgs. for pitting – Dweet or sw. o. sdlgs. for oak leaf patterns	
Exocortis	– 861-S-1 citron. A clonal bud forced on a vigorous rootstock (ke., ro. le.)	– Electrophoritic gels – mech. transmitted to Gynura for certification
Greening	– Sdlgs. of Ponken mand., Madam Vinous sw. o. or Orlando tangelo-bud transmitted or phyllid transmitted	– Flourescent markers in fruit
Impietratura	– Young gft. tree for fruit symptoms. – Seedlings of Dweet, Mand. or sw. o. for oak leaf patterns	
Infectious variegation	– Citron, lemon or sw. o. sdlgs.	– Mech. trans. to cowpea
Psorosis-A	– Sw. O., Dweet, lemon, citron or Mexican lime sdlgs; – psorosis-B lesion challenge in sw. o. sdlgs.	
Ringspot (sporosis)	– Duncan fgt., sw. o. sdlgs.	– Mech. trans. to *Chenopodium quinoa*
Satsuma dwarf (citrus mosaic) (Novel infectious mottle) (Natsudaidei dwarf)	– Citron, lemon, satsuma or other mand. sdlgs.	– Mech. trans. to cowpea or white seseme – ELISA
Stubborn	– Side or leaf graft to madam vinous sw. o.	– Culturing *Spiroplasma citri* in a prepared media – Leafhopper vector to Vinca or other weed hosts – ELISA
Tristeza	– Mexican lime, *Citrus excelsa* or *C. macrophylla* sdlgs.	– ELISA, S.D.S. – Immunodiffsuion, inclusion bodies – Electron microscope – Fluorescent antibodies – dsRNA gel
Tristeza (seedling yellows and stem pitting forms)	– Gft., sour o. and lemon sdlgs. for seedling yellows – Gft. and Madam Vinous sw. o. for stem pitting	– dsRNA gel
Tatterleaf	– *C. excelsa*, Rusk or other citrange sdlgs.	
Vien entaion	– Mexican lime or sour o. sdlgs.	

[1] Mandarin (mand.), sweet orange (sw. o.) rough lemon (ro. le.) trifoliate (trif.) grapefruit (gft.), Dweet tangor (Dweet). Mechanical transmittion (mech. trans.); seedlings (sdlgs.)

indexing tools. However, there are certain limitations in any antibody technique which should be recognized. A most critical consideration is that citrus viruses usually exist in very low concentrations in the tree, may be irregularly distributed, or be temporarily absent during periods of warm summer temperatures. 'Viruses' can be missed by ELISA. A prime advantage of the biological test using plants is that even a single or a few virus particles may replicate in the indicator plant and will show its presence as symptoms in the new flush of growth. At present, the plant should still be used for critical evaluation of heat-treated or shoot-tip grafted budwood and for indexing of important foundation blocktrees.

A recent technique for testing for presence of viruses in citrus is by the detection of double-stranded RNA using electrophoresis on polyacrylamide gels. This technique is currently being studied for separate analysis of tristeza and seedling yellows and perhaps stem-pitting triseza[9]. It has had marked success in detecting avocado viruses[8,13], and the possibilities of its use for identification of other citrus viruses is being explored.

Budwood Certification Programs

Once clean stock is produced, a program for its long-term maintenance and for the distribution of certified budwood to growers must be developed and financed. Certification programs will be varied depending on a countries specific needs. The programs in Spain[23], California[18] or Florida[6] may be studied and used as models. Basically they involve a nucleus or bank of select, virus-free, indexed, and true-to-type mother or foundation trees from which buds are collected for increase in a certified increase block. These increase blocks are usually maintained for a limited period of 18 months to 3 years to protect against chance off-type mutations and possible reinfection. New collections are repeatedly made from the nuclear or foundation block trees for new budwood increase blocks. Mother trees should be continually indexed for the presence of 'viruses'.

Buds from the increase block trees may be distributed to the grower or may be used to grow certified trees by the government for distribution to growers. There are many problems in developing, maintaining and protecting a foundation planting. Prevention of reinfection by insects or by man via mechanical transmission is foremost. Also, if tristeza-free budwood is produced in tristeza endemic areas there is the question of what protective isolate or strain should be reintroduced for cross protection.

A decision to develop a foundation block with its supportive indexing facility must be well supported and adequately financed as a long-term program. There should be policies for education and publicity to inform growers and the public of the dangers of viruses and the benefits of virus-free stock. Other considerations which must be faced are adequate facilities, government regulations, quarantine provisions, isolation, grower involvement, trained technical personnel and the logistics associated with budwood collection, storage, record keeping and means of budwood distribution to growers. The long-term positive benefits from avoidance of viruses in propagative budwood by a good certification program make all efforts worthwhile from both the economic and pathological points of view.

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CITRUS TRISTEZA VIRUS ISOLATED FROM ACID LIME IN THAILAND

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SUMMARY

Citrus tristeza virus (CTV) was isolated from naturally infected acid lime plants in Thailand. The virus was identified on the basis of host range, symptomatology, particle morphology and transmission. Host range of CTV was limited to 14 plant species in the family Rutaceae. The virus caused cup leaf, vein clearing and vein corking symptoms in acid lime. It is transmitted by aphids, Toxoptera aurantii and T. citricidus, in a non-persistent manner, and by grafting. Partially purified preparations and leaf-dip samples contained threadlike particles of about 1,700–2,000 nm in length.

摘　要

於泰國自然感染之酸檸檬植株中分離出柑桔南美立枯病毒(CTV)。此一病毒係由其寄主種類、病徵、病毒形態與傳播特性而加以判定。CTV之寄主只限於芸香科中之14種植物。酸檸檬感染後造成杯狀葉，葉脈透化與木栓化之病徵。其係經由蚜蟲(*Toxoptera aurantii* and *T. citricidus*)及嫁接感染。病毒顆粒在部分純化及葉液之樣品中爲線形，長度約1700～2000 nm。

摘　要

タイ國の自然感染したmanao(acid lime)からウイルスが單離され、そのhost range、病徴、粒子の形態、傳搬様式からcitrus tristeza virusと同定された。そのhost rangeはミカン科の14種の植物に限られ、manao上でさじ状葉、葉脈透明、葉脈コルク化などの病徴をていした。アブラムシ、*Toxoptera aurantii*および*T. citricidus*によって非永續的に傳搬され、また接木によって傳染する。部分純化およびleaf-dip標品の中に約1,700～2,000 nmのひも状粒子が觀察された。

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INTRODUCTION

Citrus cultivation originated in tropical and subtropical regions of South-East Asia, later being distributed all over the world. Thailand is very suitable for citrus growing, the commercial varieties are mandarin (*Citrus reticulata*), pummelo (*C. grandis*), and acid lime (*C. aurantifolia*), grown for domestic and export markets. Recently, diseases and pests have decreased production, tristeza being the most serious it threatens the future of the industry. First described in South America in the early 1900's, since then, it has been reported in various South-East Asian countries: India[7], Taiwan[15], Indonesia[8], and the Philippines[4].

Tristeza symptoms were found in Thailand in 1971 on acid lime in Thayang District, Phetchaburi Province as a severe infection[16], and later identified as tristeza disease caused by citrus tristeza virus (CTV) by Kunakorn in 1982[14]; however, the virus has not been studied in detail. This paper reports the results of an investigation made to characterize the CTV isolated from acid lime in Thailand.

MATERIALS AND METHODS

Virus Source and Maintenance

The virus was isolated from naturally infected acid lime plants collected from central Thailand in 1971, and maintained by successive graft transmission in an insect-proof house at Bangkhen, Thailand.

Host Range

Host range of CTV was determined by graft inoculation to 14 plant species in the family Rutaceae. Virus infection was confirmed by back grafting to healthy acid lime plants. All inoculated plants were maintained in the greenhouse for at least three months, during which an insecticide was sprayed periodically.

Virus Transmission

Seedlings of acid lime used for transmission tests were two years old. Leaves and bark tissues from new growth of systemically infected acid lime plants were used to prepare the inocula.

Mechanical inoculation was made by a procedure modified from that of the knife-cut used for exocortis[11]. The petioles and branches of receptor plants were cut (20 cuts per plant) individually with freshly contaminated shears. The shears were contaminated by cutting infected manao branches or stems. Inoculated plants were then kept in the greenhouse where pests were carefully controlled.

Aphid transmission test was done with virus-free aphids, *Toxoptera aurantii*, and *T. citricidus*, reared on healthy acid lime plants. The aphids were starved for about three hours and allowed an acquistion access of one hour on infected acid lime plants. They were then transferred to healthy acid lime plants (30 aphids per plant) and allowed a one-hour access inoculation feeding. Feeding of aphids was terminated by spraying with an insecticide.

Graft transmission test was done by leaf grafting and side cleft grafting from diseased to healthy acid lime plants[5].

For dodder transmission test, virus-free dodders, *Cuscuta* sp., were allowed to establish on infected acid lime plants for one week. After

that dodder stems attached to CTV – infected acid lime plants were trained to healthy acid lime plants and they were allowed to establish there for another one week. The dodder stems were then removed after inoculation.

Seed transmission test was attempted by planting seeds from CTV infected acid lime plants in wooden boxes containing sterilized soil. The plants were kept in the insect-proof greenhouse for about 10 months.

Virus Purification

The virus was partially purified according to the procedure modified from those described by Bar-Joseph *et al.* in 1970 and 1972[2,3]. Frozen leaf midribs of infected acid lime plants were macerated in 0.1 M Tris-HCI buffer, pH 8.0, containing 0.1% thioglycollic acid (2.5 ml/g tissue) with a chilled mortar and pestle. After the homogenate was expressed through cheesecloth, the pulp residue was re-extracted with the same buffer and the extract was centrifuged at 4,200 g for 10 min. The aqueous phase was recovered, then polyethylene glycol 6,000 (PEG) and sodium chloride were added to give a final concentration of 4 and 0.8%, respectively. After stirring for 30 min, the mixture was centrifuged at 15,000 g for 25 min. and the precipitates were dissolved in 0.1 M potassium phosphate buffer, pH 7.4, and clarified by centrifugation at 4.200 g for 10 min. The PEG purification and clarification were repeated, then the preparation was centrifuged at 34,000 g for 30 min. in tubes cushioned with 5 ml of 50% sucrose. The virus-containing zones were drawn out with a syringe, pooled and concentrated by centrifugation at 77,000 g for 1 hr. Virus pellets were resuspended in 0.1 M potassium phosphate buffer, pH 7.4.

Electron microscopy

Samples for electron microscopy were mounted on carbon-stabilized, formvar-coated grids and stained with neutral 2% phosphotungstic acid. Observations were made with a Hitachi H-300 electron microscope.

Detection of CTV was done by leaf-dip method[6] and Derrick method of immuno electron microscopy techniques[9]. The antiserum against CTV from Japan was applied in the latter method.

For ultrathin sections, tissues of 1 x 3 mm in size from midribs of CTV-infected acid lime plants were fixed with 2.5% glutaraldehyde. They were post-fixed in 2% osmium tetroxide, dehydrated in an acetone series, and embedded in a Spurr's resin. Ultrathin sections were cut using glass knives mounted on a Porter-Blum MT-2B ultramicrotome, and stained with 4% uranyl acetate and 2% lead citrate[12,17].

RESULTS

Host Range

All tested plants were infected with the virus. Reactions of the plants to the infection are summarized in Table 1. Infected *Citrus aurantifolia* and *C. hystrix* showed cup leaf, vein clearing and vein corking symptoms. *C. aurantium* and *C. madurensis* showed leaf chlorosis and stunting, *C. excelsa* showed vein corking and vein yellowing, *C. limon* showed small leaf and stunting, and *C. sinensis* showed leaf chlorosis.

Aegle marmelos, Feronia limonia, F. lucida, C. grandis, C. limettiodes, C. paradisi x *C. reticulata* and *C. reticulata* did not develop symptoms, but electron microscopic observations and back-inoculation tests indicated that they were systemically infected with the virus without visible symptoms.

Symptomatology

Symptoms in field on grown acid lime plants were vein-clearing in young leaves, die-back of young twigs, thickening and malformation of leaves and reduction in size and number of fruits. In addition, the quick-decline tristeza symptoms of leaf and fruit dropping, wilting and death of plants were observed in some growing areas.

Virus Transmission

CTV was transmissible by grafting and by the two aphid species, *Toxoptera aurantii* and *T. citricidus*, in a non-persistent manner (Table 2). Inoculated acid lime plants showed symptoms similar to those described above about six weeks after inoculation. The virus was not mechanically transmissible by the modified knife-cut inoculation. Furthermore, it was neither transmitted by dodder (*Cuscuta* sp.) nor through seeds (Table 2).

Table 1 Host reactions to infection by citrus tristeza virus

Tested plants			Symptoms[a]
	Species	Common names	
Aegle	*marmelos* Corr.	bael fruit	S
Citrus	*aurantifolia* Swing.	acid lime	VCl, VCo, CL
C.	*aurantium* L.	sour orange	Chl, St
C.	*excelsa* Wester	—	VY, VCo
C.	*grandis* Osb.	pummelo	S
C.	*hystrix* DC.	leech lime	VCl, VCo, CL
C.	*limettiodes* Tanaka	Palestine sweet lime	S
C.	*limón* Burm. f.	Lisbon lime	SL, St
C.	*madurenis* Loureiro	Calamondin	Chl, St
C. paradisi Macf x *C. reticulata* Blanco		Orlando tangelo	S
C.	*reticulata* Blanco.	mandarin	S
C.	*sinensis* Osb.	sweet orange	Chl
Feronia limonia Swing.		wood apple	S
F.	*lucida* Scheff.	—	S

[a] Key to symptoms: Chl = chlorosis, CL = cup leaf, S = symptomless systemic infection, SL = small leaf, St = stunt, VCl = vein clearing, VCo = vein corking, VY = vein yellowing

Table 2 Transmission of citrus tristeza virus by aphids (*Toxoptera aurantii* and *T. citricidus*) dodder (*Cuscuta* sp.), graft, mechanical (modified knife-cut inoculation) and seed

Types of transmission	Rates of transmission[a]
Cuscuta sp.	0/9
graft	10/10
modified knife-cut inoculation	0/10
seed	0/1017
Toxoptera aurantii	5/10
T. citricidus	7/0

[a] number of infected plants/number of tested plants

Virus Purification

Two bands, green colored and opaque ones were observed at the interface between two layers of the preparation and sucrose after the high speed centrifugation. The opaque band contained threadlike particles with an average length about 2,000 nm (Fig. 1) when observed under the electron microscope.

Electron microscopy

Electron microscopy of leaf-dip samples prepared from acid lime leaf tissue infected with CTV revealed threadlike particles with an average length (79 particles) of 1,700-2,000 nm (Fig. 2). Particles observed from IEM-Derrick and ultrathin section preparations were similar in size.

DISCUSSION

Results from our studies on host range, symptomatology, particle morphology and transmission of the virus isolated from the infected acid lime. Plants in Thailand indicated that the virus is CTV. This virus is destructive especially in acid lime and mandarin[1] and found widely distributed in all commercial citrus varieties[1,13]. The wide distribution of CTV could be attributed to the existence of the inoculum source and the high population level of the efficient vector, *T. citricidus*.

Most citrus plants in Thailand are propagated by airlayers. One way to reduce the destruction caused by this virus is to remove CTV-infected plants once they are detected and replace them with new airlayers from virus-free plants. Presently, it is possible to get acid lime and pummelo plants which are CTV-free, although a certain number of these CTV-free plants were previously obtained for the application of the shoot-tip grafting *in vitro* technique[1]. Another way to reduce the destruction caused by CTV is to develop an effective control of the insect vector. Further, pre-immunization of CTV-free plants should be considered for control of this virus.

In that these experimental results indicate

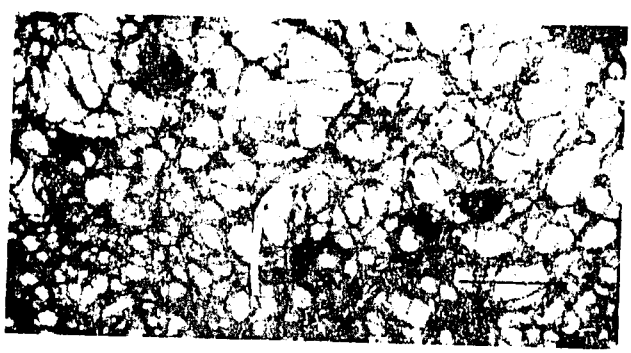

Fig. 1 Electron micrograph of partially purified citrus tristeza virus, negatively stained with 2% phosphotungstic acid, pH 7.0 Bar = 600 nm.

Fig. 2 Electron micrograph of a negatively stain ed dip preparation from diseased manao. Bar = 500 nm.

that CTV was neither transmitted by the modified knife-cut inoculation nor by dodder, *Cuscuta* sp., our results disagree with those reported by Garnsey *et al.* and Weathers and Harjung[10,18]. These CTV transmission mode studies are inadequate and need to be confirmed. Furthermore, additional purification of CTV by sucrose density gradient centrifugation and serological studies of this virus will be undertaken in our laboratory.

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CITRUS GREENING *(Likubin)* AND SOME VIRUSES AND THEIR CONTROL TRIALS

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ABSTRACT

In Taiwan greening disease, likubin, and citrus tristeza virus diseases are recognised as serious citrus diseases. Citrus tatterleaf virus (TLV) infection is endemic in much of Taiwan's citrus and has been misdiagnosed as scion/rootstock incompatibility for Ponkan and Tankan on trifoliate rootstock. An intergrated national program for the control of greening and virus diseases of citrus has been in operation since 1981.

摘　要

立枯病(likubin)與柑桔南美立枯病(citrus tristeza virus diseases)爲臺灣嚴重之柑桔病害。柑桔破葉(黃圈)病毒(TLV)爲臺灣地區之病毒，曾被誤認爲椪柑與桶柑嫁接於枳殼根砧後，穗砧不親和性所造成。一個全國性的柑桔立枯病與病毒綜合防治計畫已於1981年成立。

摘　要

台湾ではリクビン(greening)とトリステザウイルスが柑橘の２大病害である。台湾の柑橘の大部分の樹にcitrus tatterleaf virusが入つており、ポンカンおよびタンカンとカラタチ台との生理的不和合性として以前誤認されている。柑橘のリクビン病及び諸ウイルス病に對する國家的總合防除計畫が1981年から始まつている。

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INTRODUCTION

Citrus, planted to 37,000 ha, is Taiwan's most important fruit crop. The virus-like greening disease, known locally as *'likubin'* has been devestating commercial citrus varieties, Ponkan mandarin, Tankan, Liucheng sweet orange, Wentan and Peiyu pomelo, and Eureka lemon (Kinqua is and exception) since 1955. The low island-wide citrus yield of less than 15 tons/ha has been attributed to the short tree lifespan, less than ten years, due to greening infection. Additionally, Ponkan, Tankan and Liucheng are commonly infected with citrus tristeza virus causing stunting and misshapen fruits of poor quality. Recently, citrus tatterleaf virus which shows no visible symptoms has been detected as endemic in trees rooted on Sunki or Rangpur lime stocks. This latent infection is assumed to contribute to reduced growth vigor.

GREENING *(Likubin)* DISEASE

The causal agent of greening, *likubin*, 'LK', also know as yellow shoot, has been widely studied since 1955. It is caused by a walled, fastidious procaryote limitted in phloem. Bove (1980) considered the agent to be gracilicute-like. Broad-wide electronmicroscopic study has shown the agent to be pleomorphic filamentous bodies of a range of thickness (50-940 nm in diameter), surrounded by a 20-35 nm thick envelope consisting of outer layer and inner unit membrane. The morphological life cycle of the pathogen and the population dynamics in the host plant were investigated over one year. The highest population of LK bodies was detected in sieve tubes of diseased Ponkan trees while lower contents were found in Tankan and Liucheng trees. Fewer sieve tubes in diseased pomelo trees contained the bodies but the infected sieve elements harbored considerable numbers of the LK bodies. The LK bodies were detected year round; however, the population was highest in fall and winter, and lowest in spring with gradual increase during summer. The number of mature, large, electron-lucent bodies seen in the sieve elements was greater during winter.

Greening infected trees commonly had mix-infection with CTV. The LK-procaryote-like organism (PLO) showed dominance over CTV, since the ELISA values with the LK-diseased materials were generally lower than the those of the samples collected from healthy-looking CTV-infected trees. The LK-agent was transmitted by 4-5 instar nymphs and adults of psylla, *Diaphorina citri*, at very low transmission rate (5/380).

On this Asian citrus psylla, so far endoparasites[11], *Psyllaephagus* spp., have been found ineffective in biological control. In order to lower the psyllid vectors, the ectoparasites, *Tetrastichus dryi* and *T. sadiatus*, are expected to be introduced to Taiwan as biological controls[7].

Tetracyclines including achromycin and terramycin showed therapeutic effectiveness against the LK-agent by scion dipping treatment[27]. Temporary remission of symptoms occured in LK-agent diseased trees injected with tetracycline solution (1,000 ppm/1-2ℓ), and the effectiveness of antibiotic infusion could be improved by pruning diseased twigs just after injection[26]. Tetracycline was first detected in leaves two days following injection, its activity increased to a maximum at 6-8 days and then declined gradually to become non-detectable at 12-22 days[4].

Research for Control Measures

a) Preparation of monoclonal antibodies against LK-FB has been attempted by using hybridoma technique. The LK-FB was to propagated to high population levels in dodder from which LK-FB preparation was obtained and used as antigen for immunizing Balb/c mice and screening hybridoma lines. Some hybridoma lines showed secreating monoclonal antibodies specific to LK-preparation. Further investigations are still under way.

b) Epidemiological study on the infection period by psyllid vector is in prograss.

MAJOR VIRUS DISEASES OF CITRUS IN TAIWAN

Citrus tristeza virus complex with a seedling-yellows component (CTV-SY) has been commonly found in greening affected trees of the commercial varieties except pomelo in Taiwan. The greening disease had been misdiagnosed as caused by CTV-SY during 1960s[15]. However, CTV-SY was also detected in healthy looking citrus trees. Ponkan, Tankan and Luicheng rooted on Sunki currently cultivated on this island, are tolerant to CTV.

Prior to 1978, CTV caused no serious disease problem. The prominent 'dwarf' disease was first noticed to affect 'Miyu', 'Peiyu' and 'Wentan' pomelo, and grapefruit in some isolated areas in 1978. The disease is characterized by bunchy appearance of new shoots with leaf curling and atrophy, internode shortening, severe stem-pitting, stunting, and misshapen fruits with thick peel. The dwarf disease was attributed to a strain (CTV-D) of CTV distinct from the current strains of CTV-SY. Grapefruit and pomelo seedling infected with this dwarf strain (CTV-D) showed higher ELISA values than those of the same citrus seedlings inoculated with common isolates of CTV-SY[22]. Recently, it was found that CTV-SY caused obvious symptoms in pomelo seedling 2-3 months after inoculation; however, the symptoms disappeared gradually thereafter, and finally no CTV could be detected from the inoculated pomelo one year later. This suggested a reason why no CTV was normally detected in the pomelo orchard trees on this island. Nevertheless, CTV-D was retained in pomelo seedlings. In general, stunted but otherwise healthy looking trees in orchards showed high ELISA values, while low or no ELISA values were detected in tall healthy looking citrus trees. Presumably, CTV strains play an important role in lowering yield and quality of citrus fruits on this island. Therefore, preimmunization with mild protective strain has been tried on healthy seedlings derived from foundation virus-free stocks to prevent reinfection by severe strains of CTV. Studies on cross protection continue. Attempts are being made to produce monoclonal antibodies against mild protective strains or severe strains to monitor the effectiveness of the cross-protection in the field.

Citrus tatterleaf virus (TLV) is a citrus virus causing 'tatterleaf' of 'Kalpi' lime *(Citrus excelsa)* which was first found in the 'Meyer' lemon introduced to California from China[29]. Calavan *et al.*[3] demonstrated such severe reactions as bud union creases and stock reduction of Satsuma mandarin or sweet orange on Troyer citrange and trifoliate orange rootstocks graft-inoculated with tatterleaf virus infected Meyer buds. Miyakawa and Matsui[17] reported that the abnormal bud union of Satsuma mandarin on trifoliate orange was similarly due to the virus causing tatterleaf virus. Recently, Miyakawa[16] detected TLV in Ponkan and Tankan mandarin and pomelo formerly introduced from China mainly from Taiwan.

The fact that Ponkan and Tankan trees rooted on trifoliate were severely stunted had been assumed due to scion/rootstock incompatibility in Taiwan. Accordingly, Sunki mandarin has been cultivated as rootstock showing suitable growth with the scion cultivars Ponkan, Tankan and Luicheng sweet orange. When preliminary indexing of TLV on budwoods both of healthy looking and greening affected citrus trees was made using the indicator plant, *C. excelsa*, provided by Dr. Calavan, in most cases no distinct leaf symptom of tatterleaf was demonstrated in the bud-inoculated *C. excelsa* seedlings which did

however, show severe vein clearing, leaf chlorosis, and stunting presumably due to CTV-SY in mixed infection. The widespread presence of CTV-SY in Taiwan citrus trees made it difficult to detect TLV using the *C. excelsa* indicator. This difficulty was overcome by using Rusk citrange instead of *C. excelsa* as indicator. About 70% of citrus trees grown over the island have been indexed as infected with TLV by top-grafting Rusk citrange buds onto rough lemon seedlings inoculated with suspect citrus buds[29]. The healthy looking orchard trees of Ponkan, Tankan, and Liucheng rooted on Sunki rootstocks, were commonly found to be infected with TLV by indexing with *C. excelsa*, Rusk and Troyer citranges, and *Chenopodium quinoa*. Greening affected citrus trees also contained TLV. Rusk citrange buds top-grafted on Liucheng sweet orange, Peiyu pomelo or rough lemon seedlings had the most sensitive reaction to TLV producing chlorotic spots and ragged margins on distorted leaves of Rusk citrange shoots 4-6 weeks after graft-inoculation with buds from infected trees. On the other hand, Troyer citrange seedling or Rusk citrange buds top-grafted on Troyer citrange seedlings only showed mild leaf blotching or no visible leaf symptoms except stunting 3-6 months after bud-indexing. The TLV in the scion cultivars including Ponkan and Tankan, sweet orange grapefruit and pomelo, was readily eliminated through heat therapy at 40/30°C (16/8 hr. photoperiod) over three months or shoot-tip grafting. Various isolates of TLV produced different degrees of typical symptoms in Rusk citrange shoots. This implies that there are several strains of TLV in Taiwan.

In the preliminery test, TLV caused considerable stunting of Troyer and trifoliate seedlings, or of seedlings rooted on Troyer or trifoliate.

The virus was transmitted mechanically, however, transmission trials with aphis *Toxoptera citricidus* have revealed negative results so far. The virus was purified from *C. quinoa* systemically infected with TLV. The virus particles were flexuous rods about 650 nm in length, 12-13 nm thick, and 3-3.5 nm cross banding pitch. Particle morphology and other biological data enable us to classify TLV as a carlavirus. For a rapid indexing method with the ELISA test, preparation trials using the virus preparation from *C. quinoa* for immunizing Balb/c mice have been made through the hybridoma technique. Serological work is in progress.

Trifoliate orange and Troyer citrange can be used as the rootstock again for improving the quality of fruit now that TLV can be eliminated from scion cultivars through a modified method of shoot-tip grafting and heat-therapy.

Citrus exocortis viroid produces exocortis symptoms of bark-shelling and splitting noticed in a few cases on Rangpur lime rootstock top-grafted with Valensia sweet orange in Taitung area. The indexing with Etrog citron (861-S) was made by graft-inoculation with suspect buds. A small number of buds derived from field sweet orange trees caused a positive reaction showing rolling of citron leaves, while samples from Ponkan, Tankan and pomelo trees had negative responses.

INTEGRATED CONTROL OF GREENING AND VIRUS DISEASES OF CITRUS

The following integrated control measures have been proposed to combat the virus and virus-like diseases of citrus under the national program of citrus industry improvement supported by the Council of Agriculture since 1981.

1) Establishment of healthy foundation stocks through a modified method of shoot-tip grafting, heat-therapy or nucellar line selection (except pomelo), and a rapid propagation method of budwood.

2) Cultivation of healthy citrus seedlings through the three stem system under a budwood certificate program.

3) Elimination of greening diseased trees to prevent reinoculation.

4) Protection of healthy citrus seedlings grown in clean fields as follows:

   a) Spraying of insecticide (Dimethoate or Durshan-M, 1,000X) at 10-20 day intervals during critical infection periods to prevent

reinfection with the LB-agent.

b) Preimmunization of healthy foundation stocks or mother trees with protective mild strains of CTV.

c) Disinfection of TLV or exocortis viroid contaminated pruning knifes, saws or scissors by dipping in 1% NaOCl (10X bleach) and rinsing with 2% summer oil + 5% acetic acid (vinegar).

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CITRUS TRISTEZA VIRUS: IMPACT AND CONTROL BY PREINOCULATION IN JAPAN

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SUMMARY

In Japan, citrus tristeza virus (CTV) and its primary vector have been widespread, but unidentified before the study made on Hassaku dwarf. Injury caused by CTV has been defined on Hassaku, Natsudaidai, Iyo, sweet oranges, some buntans, Yuzu, tangelos and tangors; affected trees decline with severe stem pitting and yield small irregular shaped fruit. Severe strains of CTV-SP or CTV-SY are responsible; however, a large number of satsuma mandarin trees are symptomless carriers of these severe strains. A healthy Hassaku HM-55, found by intensive field surveys, was noted to carry mild forms of CTV-SP and vein enation virus (CVEV) in complex, and was protective against severe strain. Preinoculation of mild isolates including CTV-SP, CVEV or unknown components are also protective, but their effects depend on the host plant species.

摘　要

日本之柑橘南美立枯病毒(CTV)及其主要媒介昆蟲已相當普遍，但其造成之傷害程度，在Hassaku矮化症研究前，並未被確定。CTV會在八朔、夏橙、伊予、蜜柑、某些文旦、柚子與橘子中造成傷害。感染植株減產，著生小而異型之果實。重症型CTV-SP或CTV-SY之系統均可造成上述徵象。許多Satsuma柑橘砧穗受重症型病毒感染，但不表現病徵。經田間廣泛調查，找到一株健康之Hassaku HM-55之植株，已知受到輕症之CTV-SP與脈瘤病毒（CVEA）複合感染，因而對重症病毒表現抗性。預先接種CTV-SP、CVEV或輕狀病毒，亦顯現交互保護效果，但其程度則依寄主之種類而有所不同。

摘　要

日本ではトリステザウイルスとその媒介昆蟲は廣く分布しているが、その實害はハツサク萎縮病の研究がおこなわれるまで明かでなかつた。トリステザの被害はハツサク、夏ダイダイ、イヨカン、スイートオレンヂ、或種のブンタン、ユズ、タンジェロ、タンゴールで明かに現れる。被害樹は激しいステムピツティングを伴つて衰弱し、小さい不定形の果實を著ける。CTV-SP又はCTV-SYの強毒系統が病氣をおこす。温州ミカンの大部分はこれらの強毒系統の無病徵保毒者である。集中的に行つた圃場調査の結果發現されたハツサクHM-55はCTV-SPの弱毒系統とvein enation virus (CVEV)の2つをもつており、強毒系統の感染を免れていた。CTV-SPの弱毒系統、CVEV又は未知の成分を含むものの前處理は強毒系統の感染を防ぐがその效果はミカンの種類により異なる。

CITRUS TRISTEZA VIRUS: IMPACT AND CONTROL BY PREINOCULATION IN JAPAN

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INTRODUCTION

The first tristeza disaster was recorded in 1930s in South America, however, its causal virus originated in Asia and broke out with trade of citrus plants caused by worldwide botanic and commercial interest in the cultivars coupled with world trade development[1]. Citrus tristeza virus (CTV) is widespread in most citrus growing areas in the world.

In Japan, CTV and its primary vector, *Toxoptera citricida* Kirk., were widely distributed, but unnoticed till the Hassaku dwarf was recognized as a CTV-induced disease[24,25]. Since satsuma madarin grafted on trifoliate orange trees, a tolerant combination to the virus, had occupied 80 to 87 percent acreage the disease was regarded as of minor importance. However, overproduction of satsuma mandarin and changes in consumers tastes resulted in variety renewal with CTV-susceptible varieties. This paper deals with symptoms on various citrus varieties caused by CTV, the virus strains distributed in fields and some trials to control the disease by preinoculation with mild strains in Japan.

CTV IN JAPAN

CTV causes different symptoms on citrus plants depending on virus strain, the variety of host plant and scion-rootstock combination. In Japan, most citrus trees are on trifoliate orange, *Poncirus trifoliate* (L.) Raf.. Trifoliate orange-rootstock trees are highly tolerant against quick decline or tristeza, but still susceptible to stem pitting disease[2,26].

Intensity of stem pitting was surveyed on various citrus plants at the Okitsu Branch, Fruit Tree Research Station (FTRS)[28]. Cultivars or varieties which developed severe stem pits were Fingered citron *(C. limonimedica)*, Ponderosa *(C. pyriformis)*, most of buntans *(C. grandis)*, grapefruit *(C. paradisi)* and the relatives, Nanshoudaidai *(C. taiwanica)*, sweet orange *(C. sinensis)* and its relatives, Funadoko *(C. funadoko)*, Iyo *(C. iyo)*, Yuzu *(C. junos)* and its relatives, King *(C. nobilis)*, Tachibana *(C. tachibana)*, Calamondin *(C. madurensis)*, most of tangelos and tangors, and citrangequat. In contrast, sweet lime, lemon, most of mandarins or tangerines, trifoliate orange and citrange were nearly free of stem pitting. Satsuma mandarin had been considered free of stem pitting[26]; however, it was revealed that some of these trees showed mild stem pits[27].

In the case of self-rooted trees for breeding, not only severe stem-pits but also yellowing and subsequent decline or dieback were observed in many seedlings of Natsudaidai, various hybrids of Iyo, pummelos and Hyuga-natsu *(C. tamrana)*[12].

SYMPTOMS ON SOME IMPORTANT VARIETIES

Hassaku dwarf disease characterized by severe pitting on twigs and trunk, resulting in decline of trees and small and irregular shape fruit, has been the primary disease of Hassaku, *C. hassaku* Hort. ex Tan.. According to the field survey in Hiroshima prefecture in 1961[30]

the acreage of affected trees was 400 ha (84%) causing about 30% loss in production. A survey in Tokushima prefecture in 1969[15] revealed that 28.7% of trees were severely affected and there was a close relationship between the intensity of pitting on 2-3 year-old twigs and fruit loss. The causal virus was a severe strain of stem pitting CTV (severe CTV-SP), with no seedling yellows reaction on the test plants[16,21].

In recent years, damage caused by Hassaku dwarf disease has been reduced through variety certification programs and preimmunization. The acreage of Hassaku shifted from 4,860ha in 1970 to 9,930ha in 1983.

Natsudaidai, *C. natsudaidai* Hay., 16,300 ha acreage in 1983, is also affected with stem pitting. Field surveys in Kumamoto prefecture during 1973-1976[7] showed that five percent of Kawano-natudaidai trees were severely affected and another 80% of the trees had developed some stem pits. On the severely affected trees small-sized fruit proportionally inceased to 68%, while it remained at 30% and 10% on moderately stem-pitted tress and the normal, respectively[20] (Fig. 1). However, when Natsudaidai trees were grown in a deeply plowded field, reduction of fruit size was not so marked even if severe stem-pits developed[20]. Fruit thinning may somewhat improve the enlargement of fruit-size on the affected trees[8]. Causal virus was suspected to be a severe strain of CTV-SP[18,20].

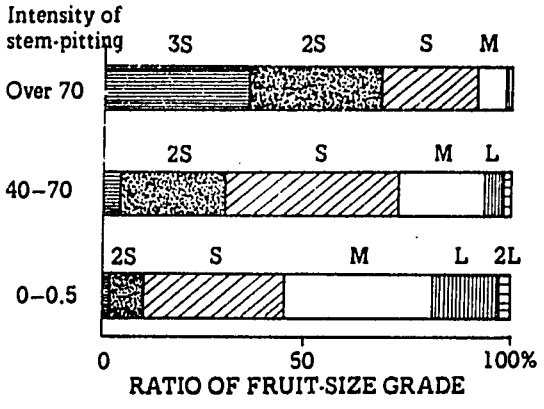

Fig. 1 Relation of fruit size to intensity of stem pitting on Kawano-natsudaidai trees[20].

Iyo has a history of stem pitting disease; brown spots or rind-oil spots appear on the fruit. A severe strain of CTV is probably causal factor for the spots. Planting of Iyo, in particular Miyauchi Iyo, markedly increased in recent years, from 2,120 ha in 1975 to 10,700 ha in 1983. Most of these were propagated by top-grafting on satsuma mandarin. This practice accelerates disease development because of infection by severe strains of CTV through the interstock, over fruiting and failure in tree vigor.

Navel orange, 4,970 ha in 1983, also has similar problems. These topgrafted trees should be replaced with nursery plants propagated from certified budlines in the near future.

Among other minor varieties, Seminole tangelo[9], Banpeiyu *(C. grandis Osb.)*[29] and Yuzu[17] also suffer severe damage from stem pitting disease. In particular, the fruit of severely affected Yuzu develop brown spots or rind-oil spots with gumming[17]. Kiyomi tangor, a newly bred and fashionable variety in Japan, shows severe stem-pits.

CTV STRAINS IN JAPAN

It is certain that various strains of CTV are present in complex in host plants[5,14]. To determine the strains present three indicator plants are used, acid lime (*C. aurantifolia* Swin.), sour orange *(C. aurantium L.)* and a composit tree of sweet orange on sour orange stock (Fig. 2)[14]. Most satsuma mandarin trees are free of CTV symptoms, but almost all of the trees are carriers of CTV[26]. An indexing[18] showed that about half of the satsuma mandarin trees carried CTV-SY which caused yellowing and stunting of sour orange or lemon, and severe symptoms on acid lime, and the other trees carried milder strains of CTV-SP which caused mild symptoms on acid lime and no symptoms on sour orange. When various cultivars of satsuma planted in the field of Okitsu Branch, FTRS were indexed, all of the stem-pitted trees carried CTV-SY which caused severe symptoms on both acid lime and lemon, of the trees free

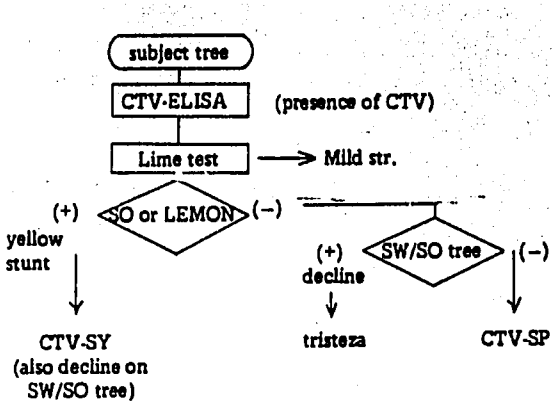

Fig. 2. A flow chart showing the determination of CTV strains by symptoms on three index plants: nucellar seedlings of acid lime (lime), sour orange (SO) and composit trees of sweet on sour orange.

of stem-pits some carried mild CTV-SP and others carried CTV-SY[27]. These results suggest wide distribution of severe strain CTV in Japan.

Indexing on the trees of various varieties of cultivars planted in the field of Okitsu Branch, FTRS[28] revealed that rough lemon, Meyer lemon, sweet orange, ponkan, tankan, most mandarins, tangerines, tangelos and tangors were carrying CTV-SY. Lemon and its relatives, grapefruit, buntan and its relatives, Natsudaidai, sour orange and citrange were carrying CTV-SP. As mentioned above, declined trees of Hassaku and Natsudaidai were carrying severe strains of CTV-SP, in contrast, apparently healthy trees but showing slight stem-pits were carrying milder strains of CTV-SP[18,20]. In the case of Yuzu, declined trees were carrying CTV-SY and apparently healthy trees were carrying milder strains of CTV-SP[17]. Since these old bud-lines have been propagated by grafting and planted in fields for many years, most of the trees irrespective of symptom are carrying various CTV strains in complex.

At the Kuchinotsu Branch, FTRS, several thousand CTV free trees of buntan, sweet orange, grapefruit and Natsudaidai were planted. In the field they were allowed to be naturally infected through aphid transmission. It was assumed that due to the small amount of virus transmitted, segregation of individual strains usually occuring in CTV complex should be possible, as infection should be by a single component. Later indexing was done at 10 to 13 years after planting, using indicator plants and enzyme linked immunosorbent assay (ELISA) with anti-CTV-SP gamma globulin[12] as shown in Fig.2.

Of the buntan trees indexed, severely stem pitted and somewhat dwarfed trees were carrying CTV-SP. Some buntan trees with yellowing and declined but not stem-pitted were carrying CTV strains which induced very mild symptoms on both acid lime and lemon. This result suggests the presence of seedling yellows component in the virus complex coupled with a mild CTV-SP strain.

Of the grapefruit seedlings severe stem pitting and dwarfed trees were observed. These trees were infected with a tristeza strain which induced decline of sweet orange on sour orange trees but did not induce yellowing in lemon seedlings.

Of sweet orange CTV-SY was detected in the declined trees and also found to be common in apparantly healthy and vigorous trees which had some stem pitting. Where a sweet orange showed severe stem pitting and dwarfing, it was found to carry a severe strain of CTV which induced extreme stunting, leaf curl and mosaic symptoms on grapefruit but did not induce yellowing in lemon. This fact suggests that the decline of sweet orange trees is not directly related to the presence of CTV-SY.

Of the Natsudaidai trees, healthy trees carried mild CTV-SP while the yellowed and subsequently declined trees carried severe strains of CTV which induced severe reactions in lime but only slight stunting and yellowing in lemon. This result suggests that the seedling-yellows component in Natsudaidai disappeared and the severe stem pitting component remained.

As indicated in many investigations, the seedling-yellows component of a virus complex tends to disappear while the severe stem pitting component remains when the virus complex is passed through lemon, pummelo and sour orange[14]. The above results indicate that the CTV-SP is the strain responsible for this effect

and therefore the residual CTV-SP is important for these plants when grafted onto tolerant root stock.

CONTROL BY PREINOCULATION WITH MILD STRAINS

Field surveys in Hiroshima prefecture discovered an apparently healthy over 60-year-old Hassaku tree in 1962[21,22]. The tree called HM-55 was carrying a mild strain of CTV-SP and vein enation virus (CVEV) in complex[21]. Seedlings of acid lime and Natsudaidai preinoculated with the viruses by tissue grafting and also the nursery plants propagated from HM-55 were to some extent protected against hassaku dwarf disease (causal CTV-SP) transmitted by *T. citricida.* Their protetcion failed when severe CTV-SP was transmitted by tissue grafting or by more than fifteen aphids per tree. Propagated trees of HM-55 showed small stem pits and only 8.6% of them declined before 9 years after orchard planting, in contrast, 76.5% of similar trees propagated from old budlines declined[21,22]. Thus, HM-55 became widespread in Japan. When Noma-beni-hassaku, a bud mutation having reddish rind became fashionable, its virus free-budline was produced by heat treatment and preinoculated with viruses from HM-55 before propagation. The preinoculated budline was propagated in a screenhouse free of aphids.

To control stem pitting disease of Yuzu, preinoculation with mild strains collected from apparently healthy and old trees of Yuzu and Hassaku were effective in field trials[19]. When the preinoculated seedlings of Yuzu were indexed eight years after planting, only mild strains of CTV were detected; in contrast, non-preinoculated trees suffered infection of severe CTV-SY.

Indexing of stem-pit free trees in the orchards of Kuchinotsu Branch, FTRS revealed that some seedling trees carried a mild strain of CTV-SP or CVEV alone[13]. Some of the CVEV isolates were protective on sour orange seedlings against CTV-SY transmitted by short term contact tissue grafting[10]. Preinoculated Yuzu seedlings with an isolate of CVEV were better protected than those preinoculated with HM-55 strain, against severe CTV-SP transmitted by aphids[23].

An isolate, No. 145, collected from a pummelo seedling was protective for sweet orange or grapefruit trees on sour or trifoliate orange rootstock against CTV-SY transmitted by *T. citricida.*(Fig. 3)[11]. This isolate produced no symptoms on CTV test plants and was negative by

Fig. 3 Five-year-old potted trees of sweet orange on sour orange rootstock after inocluation with CTV-SY transmitted by *T. citricida.*

A. preinoculated with mild isolate No. 145, showing normal growth

B. not preinoculated trees, showing severe stunting of seven trees among ten

CTV-ELISA test. In the preinoculated trees, CTV concentration as detected by ELISA was very low and decreased with time. Viruses present in budwoods collected from those trees caused only mild symptoms on acid lime, and slight or no symptoms on sour orange.

However, the protective activity of mild strains changed with the host plant or scion-stock combination[13]. The isolate, No. 145 was markedly protective for sweet orange on sour orange stock (SW/SO), and for Hassaku on trifoliate orange rootstock (HA/TRIF), but less protective for Natsudaidai on trifoliate orange (NA/TRIF) trees (Table 1). A CVEV isolate was protective for NA/TRIF trees, but not protective for SW/SO trees and only slightly protective for SW/TRIF trees. The HM-55-carried viruses were protective for HA/TRIF and SW/TRIF trees, but not protective for SW/SO, and slightly protective for NA/TRIF trees. By the similar tests at Okitsu Branch, FTRS[6], some protective isolates against severe strains were obtained, with effects which were also host plant variety dependent. These mild isolates are now being given field trials on various varieties at several experiment stations in Japan.

PROBLEMS AWAITING SOLUTION

Because of variety renewal and top-grafting, various virus or virus-like diseases including satsuma dwarf, budunion crease (caused by tatterleaf virus) and exocortis have become widespread in Japan. Subsequently, the Ministry of Agriculture, Forestry and Fisheries, Japan now supports prefectural governments in establishing variety certification programs, using the ELISA technique to detect some viruses, elimination of viruses by heat treatment or shoot-tip grafting and establishment of screenhouses for the keeping of certified bud-stock trees. Elimination of virus or virus-like pathogens in some important cultivars has been successful at several experiment stations. However, virus-free stock becomes infected with severe strains of CTV immediately after field planting, from the many CTV-carrier plants (particularly satsuma mandarin) by aphid vectors. Experiments show[21], insecticide sprays or soil treatment with systemic insecticide aimed to protect virus-free trees from vectors offers little protection against CTV infection. Thus, preimmunization with a mild virus is expedient.

Protection of mild viruses against the severe strains is not complete. Important in propagation of preinoculated budline is the fact that if the bud-soruce trees were planted in a field without any control of aphids, they would be infected with severe strains in mosaic. If contaminated buds with severe strains were used for propagation, even if the stock tree showed no symptoms, the nursery trees would develop sev-

Table 1. Protection of preinoculated citrus trees with some mild strains against CTV-SY complex[13].

Isolate	Virus	GFT[a]	SO[a]	ML[a]	SW/SO	SW/TRF	HAS/TRF	NAT/TRF
No.145	Unknown	■	X	▲		O	■	X
No.1605	CVEV alone	X	■	▲	X	▲	X	■
No.1597	CTV-SP	■	■	■	X	▲	▲	X
HM-55	mild CTV + CVEV	X	■		X	O	O	▲

■: good protection O: fair protection a) Challenged by short-term contact of affected tissue. Others were transmitted by *T. citricida*

▲: poor protection X: no protection

ere symptoms in early stages. Therefore, not only original virus-free trees but also preimmunized trees and their first derivatives used for bud propagation should be grown in a screenhouse to protect against aphid vectors. This protection in propagation systems is now insufficient.

The best suited isolate of mild virus should be selected for each variety. It may take 10-20 years before the effects of the mild strains are properly evaluated by field trials. Therefore, rapid evaluation by check for contamination and population trends of severe strains in early stages is desirable; for this, analysis of double-stranded RNA[3,4] seems useful. Identification of CTV strains as mild or severe, and their seedling yellows or stem pitting component should be clarified at particle level or molecular level.

Cross protection tests suggest some relation between CTV and CVEV. Determination of particles associated with vein enation is in progress in Japan. CTV transmisibility by *Aphis gossypii* or *A. citricola* has been proved in many countries, but it has been not determined in Japan.

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DISCUSSION

Q. (C.N. Roistacher)
Do you feel optimistic about finding good resistance to most of your severe type, stem pitting and seedling yellows strains?

A. We have several strains and some of them protective against severe strains of tristeza, but the application of these protective strains may take some ten or twenty years. Now we are trying these in the field. As far as propogation of the mild strains, we have some new foundation stock inoculated and are trying these in Japan.

STUDIES ON A NEW VIRUS DISEASE OF PAPAYA IN THE PHILIPPINES

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SUMMARY

A virus disease of papaya was first observed in Silang, Cavite in the Philippines, 1982. The disease has spread rapidly to cover three provinces. Symptoms are prominent mottling, malformation and reduction of laminae, presence of oily dark green streaks and distinct ringspots on fruits.

The virus is transmitted by sap inoculation and by four species of aphids. Seed transmissibility has not been conclusively established. Infectivity is lost when infectious sap was subjected to temperatures beyond 55°C and dilutions below 10^{-2}. It is unstable in-vitro with longevity of eight hours under room temperature. The virus particles are flexous rods with particle lengths of 780-800 nm, and are associated with pinwheel and circular inclusions. The virus is probably related to papaya ringspot virus.

摘　要

感染木瓜的一種病毒，於1982年代早期，首先在菲律賓Cavite省之Silang地方發現，並迅速蔓延至鄰近三省。其病徵爲明顯之黃色斑痕、葉片畸型變小，並呈現油漫狀暗綠色條紋，果實上則出現明顯之輪點。

此病毒可由四種蚜蟲以及汁液接種傳播，但尚未證實能經由種子傳播。其病毒懸浮液，在55°C以上或稀釋至10^{-2}時，則喪失其感病力。於室温下，其活力約爲8小時。病毒顆粒爲線狀，長度約700～800 nm，罹病植物之細胞中有車輪狀或環形之內含體。此病毒被認爲與木瓜輪點毒素病毒(PRSV)有關。

摘　要

1982年代の初め、Silang州，Caviteで１種のパパイヤのウイルス病が初めて發見された。本病は急速に３州に擴がつた。病徵は著しいモザイク、葉の奇形と縮少、果實上の油狀の暗緑色條斑と著しいリングスポットを生じる。本ウイルスは汁液および４種のアブラムシによつて傳搬される。種子傳搬については結論がでていない。汁液を55°C以上にするか、10^{-2}以上に希釋したり、又は8時間以上に保存すると感染性は失われる。ウイルス粒子は屈曲したかん狀粒子で長さ780～800nm、“ pinwheel ”又は“circular”封入體を作る。本ウイルスはpapaya ringspot virus と思われる。

STUDIES ON A NEW VIRUS DISEASE OF PAPAYA IN THE PHILIPPINES

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Papaya (*Carica papaya* L.) is one of the most important fruit crops in the Philippines, ranking sixth in area and quantity of production among fruit crops grown there[10]. A total of 7,170 ha are planted to papaya with a production of 94,401 MT[1]. Southern Tagalog region supplies about 30% of the total production and area planted to papaya. Although not a major export crop, increasing demand in other countries indicates its export potential.

In the Philippines papaya diseases have been regarded as a major production constraint although till recently virus diseases have been considered of minor importance. Papaya mosaic[10] and papaya leafcurl[11] have been reported to occur in some areas of the country but they are not commonly seen at present, however, an outbreak of a locally unreported virus disease of papaya has been causing major concern among papaya farmers in the province of Cavite, a major producing area in Southern Tagalog region. A committee report on the investigation of papaya malady in Silang, Cavite, (UPLB, August 9, 1984) indicated that about 200 ha of papaya plantations were affected by the disease at varying incidence from 60 to 100% and yield losses were valued conservatively at about $300,000.

Towards the end of 1984, studies were initiated to generate more information on the disease and to characterize the virus for positive identification.

SYMPTOMATOLOGY

Field infected papaya plants at various stages of crop and disease development were collected, examined and described.

The symptoms of the disease vary according to the stage of plant and plant parts affected and stage of infection. The diagnostic symptoms are as follows:

Leaf Symptoms

Matured plants naturally infected in the field can be first recognized by faint chlorosis in the younger leaves. In a more advanced stage, the upper third of the crown becomes prominently yellowed (Fig. 1). Initial leaf symptoms are characterized by vein-clearing, prominent mottling and the appearance of readily seen chlorotic spots. Later, leaf blades are significantly reduced and margins tend to curl upward or downward (Fig. 2). Elongated oily dark green streaks develop on the petioles (Fig. 1).

Naturally infected young plants in the field and/or artificially inoculated seedlings first exhibit vein-clearing, chlorotic spots followed by mottling of the younger leaves. The succeeding leaves are severely reduced, filiform, thickened and the leaf surfaces become roughened or leathery. Infected young plants are severely retarded (Fig. 3).

Fruit Symptoms

Dark green concentric rings or green spots appear on the fruit (Fig. 2). As the disease progresses, fruit set is sharply reduced. Fruit that develop after infection are deformed and reduced in size. Latex flow is not affected but flavor and aroma of fruit are noticeably impaired.

Stem Symptoms

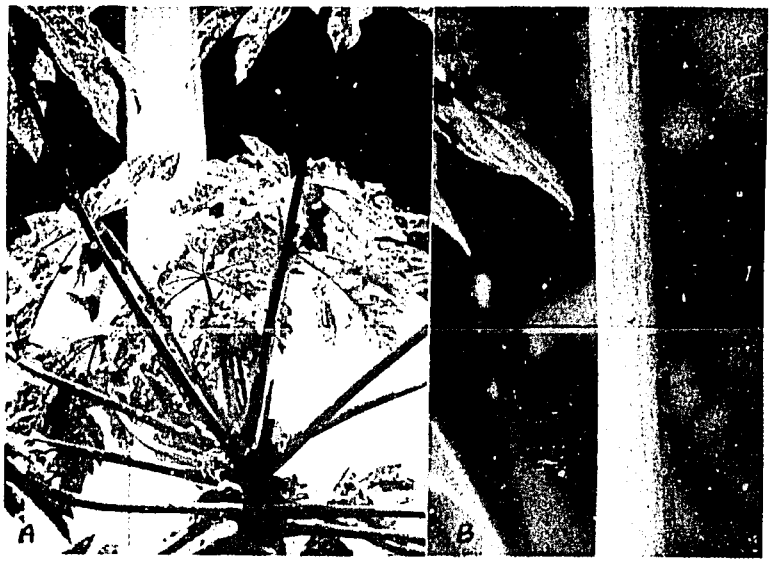

Fig. 1 Naturally infected papaya plant showing mottling, prominent yellowing of the upper third of the crown (A) and oily dark green streaks on the petioles and stem (B)

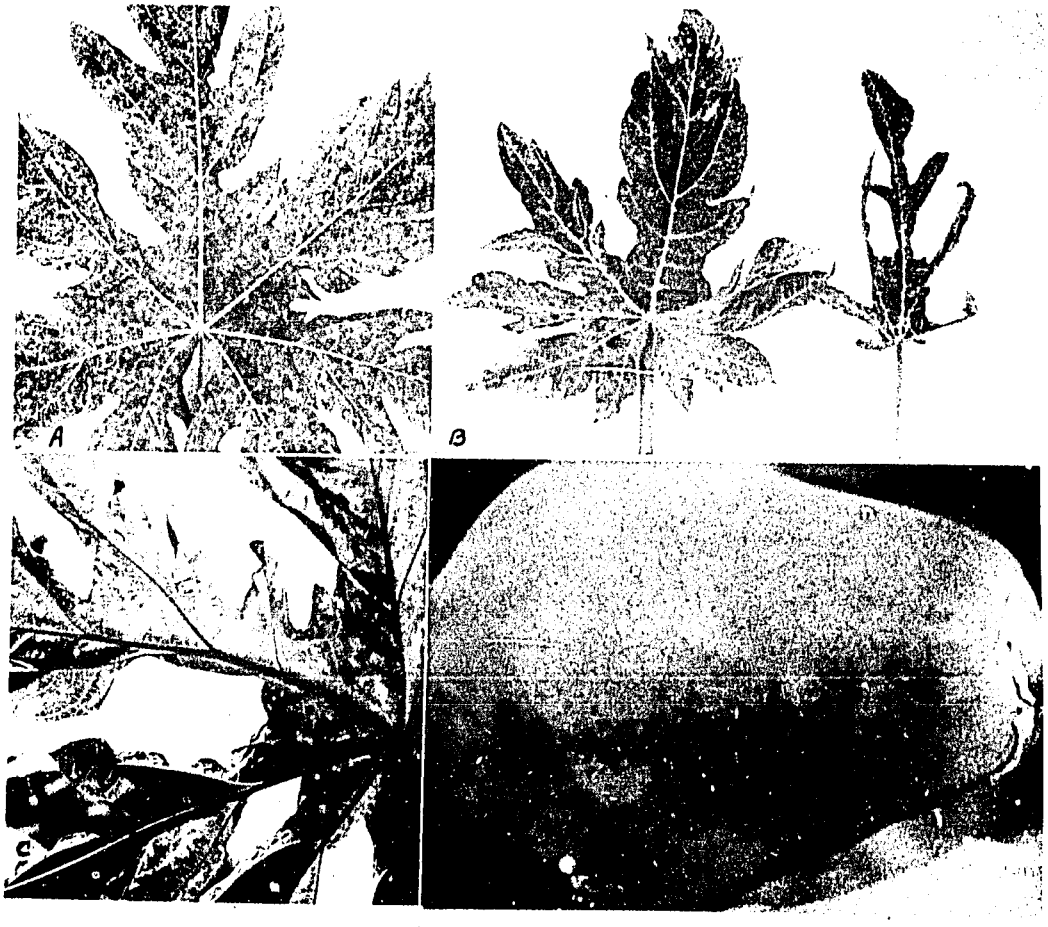

Fig. 2 Leaf and fruit symptoms of the disease: Leaf mottling and malformation, (A & B), chlorotic spots (C) and ringspots on fruits (D)

Fig. 3 Symptoms of artificially inoculated seedlings: (A) vein-clearing and (B) malformation of leaves

Stems of severely infected plants are generally shortened. Elongated green streaks or concentric rings are commonly observed on the upper portion of the stem.

TRANSMISSION STUDIES

Mechanical Transmission

Leaves showing the typical symptoms were collected and were cut into small pieces. These were macerated in 0.01 M phosphate buffer (pH 8.5) at a ratio of 1:1 W/V. Sap was immediately inoculated to one month old papaya seedlings that were previously dusted with 320 mesh carborundum.

Using the above method, the virus was readily transmitted to healthy seedlings. The first symptoms such as vein clearing and chlorotic spots appeared as early as ten days after inoculation. The typical symptoms of the disease on seedlings can be observed on the succeeding leaves 20 days after inoculation. In most cases, efficiency of sap transmission ranged from 60 to 80 percent.

Insect Transmission

Five species of insects were used in the transmission tests. These were the *Aphis craccivora, A. gossypii, A. glycines, Myzus persicae* and *Bemesia tabaci.* They were collected from *Gliricidia sepium* Stend, *Gossypium hirsutum* L., *Glycine max, Nicotiana tabacum* and *Euphorbia pulcherrima* Willd., respectively. The aphids were reared on their natural hosts under laboratory conditions and their first generation offspring were used for transmission tests. *Bemesia tabaci* was reared on healthy seedlings of sweet pepper.

The three species of aphids, after being starved for 30 min., were allowed to feed on portions of infected papaya leaves for a period of 30 min. Ten aphids were immediately transferred and allowed to feed on each healthy papaya seedling for another 30 min. *Bemesia tabaci* was allowed to feed from infected papaya seedlings

for 12 hours and 10 insects were allowed to feed in each test seedling for the same period of time. In all transmission tests, a similar number of viruliferous insects were allowed to feed on healthy seedlings to serve as check.

Results showed that the virus is readily transmitted by the four species of aphids (Table 1). *M. persicae* appeared to be the most efficient vector with transmission efficiency of 60%. Attempts to transmit the virus with *B. tabaci* were unsuccessful. None of the control checks became infected.

Table 1 Insect transmission of the virus disease 45 plants in each treatment

Insect	No. of infected plants	Transmission %
Aphis gossypii	18	40
A. craccivora	20	44
A. glycines	9	20
Myzus persicae	27	60
Bemesia tabaci	0	0

Seed Transmission

Mature fruit showing typical symptoms of the disease were gathered from various infected papaya plants. Seeds (100 to 200) were collected from each infected fruit and were sown in seed boxes placed in insect proof cages. One week after emergence, seedlings were individually transplanted in plastic cups and immediately placed in an insect proof glasshouse.

None of the seedlings grown from seeds taken from infected fruits exhibited typical symptoms of the disease 45 days after transplanting. In one trial, however, about 10% of the seedlings showed severe stunting and malformation of leaves (Fig. 4). Sap taken from these seedlings failed to infect when mechanically inoculated to healthy seedlings. These results suggest that the virus is not readily seed transmissible.

HOST RANGE STUDIES

Possible hosts of the virus were determined by mechanical inoculation as previously described. Several species of cucurbits, legumes, solanaceous crops and other plant species were used.

Repeated inoculations on various plant species indicate that the virus can only infect papaya plants (Table 2). Attempts to transmit the virus to cucurbits and other plant species were unsuccessful.

PHYSICAL PROPERTIES OF THE VIRUS

In vitro properties of the virus were determined using crude sap extracted from inoculated papaya seedlings exhibiting the typical symptoms of the disease. In all tests, one-month old papaya seedlings (Cavite special) were used as test plants.

Fig. 4 Left — controls
Right — seedlings obtained from seeds of infected fruit showing reduction and malformation of leaves and stunted growth

Table 2 Host ranges of the virus causing papaya disease in the Philippines

Host	Plants tested	Plants with symptom	Symptom[1)]
Carica papaya			
Cavite special	20	18	S
Legaspi #1	20	16	S
Solo	20	17	S
Cucurbits			
Cucumis sativus	15	0	N
Citrulus vulgaris	10	0	N
Cucurbita maxima	20	0	N
Luffa cylindrica	20	0	N
Lagenaria Leucantha	15	0	N
Legumes			
Vigna radiata	25	0	N
Glycine max	25	0	N
Phaseolus vulgaris	25	0	N
Vigna sinensis	25	0	N
Vigna sesquipedalis	25	0	N
Psophocarpus tetragonolobus	25	0	N
Solanaceous crops			
Lycopersicon esculentum	15	0	N
Solanum melongena	15	0	N
Nicotiana tabacum	15	0	N
Nicotiana glutinosa	15	0	N
Other plant species			
Chenopodium amaranticolor	20	0	N
C. quinoa	20	0	N
Datura sp.	20	0	N
Gomphrena globosa	20	0	N

1) S – systemic symptoms
N – no discernable symptom compared to the check

Thermal Inactivation Point (TIP)

Two ml of crude sap was pipetted into small tubes which were subjected to a temperature gradient from 50 to 100°C for 10 minutes and immediately immersed in ice cold water. The heated saps were mechanically inoculated to test plants.

Results indicate that the infectivity of the virus was significantly reduced when the sap was exposed to 55°C for 10 minutes (Table 3). Infectivity was totally lost when the sap was subjected to temperature beyond 55°C.

Dilution End Point (DEP)

Concentrated crude sap was subjected to a series of six ten-fold dilutions in distilled water.

Each dilution was immediately inoculated on test plants.

The test showed that the virus was still infectious at a dilution of 10^{-2}. However, infectivity was lost when the sap was diluted to 10^{-3} or beyond (Table 4).

Longevity In Vitro (LIV)

Two ml of crude sap was pipetted in screw capped test tubes and stored at room temperature (27°C). The sap was inoculated to test plants at two hour intervals for the first 12 hours and at daily intervals in the succeeding periods.

Results showed that the virus was very unstable *in vitro*. The virus was inactivated when the sap was aged beyond one day. Infection was achieved only within eight hours (Table 5).

Table 3 Thermal inactivation point of the virus (45 plants in each test)

Temperature (°C)	No. of infected plants	Infection %
50	32	75
55	9	20
60	0	0
70	0	0
75	0	0
80	0	0
90	0	0
100	0	0

Table 4 Dilution-end-point of infectious crude sap (60 plants in each test)

Dilution	No. of infected plants	Infection %
10^{0}	48	80
$10^{-1/2}$	33	56
10^{-1}	24	40
10^{-2}	15	25
10^{-3}	0	0
10^{-4}	0	0
10^{-5}	0	0

Table 5 Longivity of infectious crude sap preparation under room temperature (15 plants in each test)

Period	No. of infected plants	Infection %
0 hr	9	60
4 hr	7	46
8 hr.	2	13
10 hr.	0	0
12 hr	0	0
1 day	0	0
2 day	0	0
3 day	0	0

ELECTRON MICROSCOPY

Dipping preparations were made by dipping one side of a freshly cut 2 x 4-mm piece of infected papaya leaf in a droplet of 2% phosphotungstic acid (PTA) previously placed on a collodion-coated grid. The preparations were examined with a Phillips 410 electron microscope. Dip preparation of leaf tissues from healthy plants was also done.

Ultrathin sectioning was prepared by cutting systemically infected papaya leaves into pieces 1-2 mm wide. Tissues were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0, for 1 hr at 4°C, followed by 2% osmium tetroxide in 0.1 M phosphate buffer, pH 7.0 for 2 hr at 4°C. Following fixation, tissues were dehydrated in a graded series of acetone solutions and embedded in Spurr mixture. Sections were cut with a diamond knife and double stained by floating grids in 1% uranyl acetate for 1 hr and then lead citrate for 25-30 sec. The sections were examined with a Phillips 410 electron microscope. Pieces of leaf tissue from healthy plants were similarly treated and used as control.

Dip preparations of infected papaya leaf tissues (Fig. 5) revealed flexous rod particles. These particles were found in all the preparations from infected leaves. The particle length ranged from 780 to 800 mu. These particles

were not found in the dip preparations of healthy leaf tissue.

Ultrathin sections from infected leaf tissue also disclosed the same type of virus particles. These particles are usually found in bundles and arranged in parallel manner (Fig. 5). Ultrathin sections also revealed the presence of cytoplasmic inclusion bodies that closely resembled to the structures described as 'pinwheel' and 'circular' inclusions (Fig. 6). The inclusions were observed in all sections from infected leaves but they were not found in ultrathin sections of healthy papaya leaves.

Fig. 5 Electron micrograph of virus particles in (A) dip preparation (55,000x) and ultrathin section (48,000x) (B)

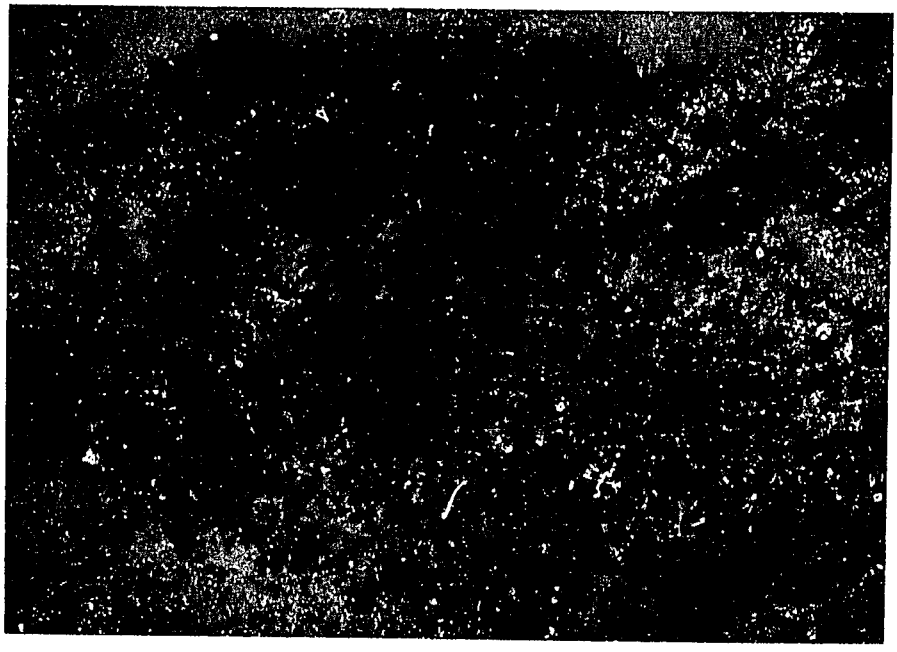

Fig. 6 Electron micrograph of inclusion bodies (A) pinwheel and circular (45,000x) (B)

GEOGRAPHIC DISTRIBUTION AND INCIDENCE OF THE DISEASE

A survey was recently conducted (April-May, 1985) in Cavite and its neighboring provinces to determine the incidence and geographic distribution of the disease. Papaya farms within close proximity of national highways, provincial and minor roads were randomly selected. The incidence of the disease was determined on the basis of symptomatology described. Personal interviews of papaya growers were also conducted to augment field data. The disease is already epidemic in three provinces, namely, Cavite, Batangas and Laguna (Fig. 7).

High incidence of the disease was confined in the municipality of Silang (Cavite) where about 80% of papaya in the Southern Tagalog region is planted. Based on interviews with papaya growers, the disease was observed in some papaya farms as early as 1982 when it did not attract attention because it was confined only to very few plants. In the early part of 1984, outbreak of the disease became apparent in farms where the disease was first observed. Recent survey indicated that the disease incidence has reached 50 to 100% in the inspected farms in the municipality of Silang. Farms with more than one year old papaya plants were affected most.

The geographic distribution of the disease has been extended to other towns of Cavite, Batangas and Laguna provinces. High incidence of the disease which range from 10-50% can only be found in areas where papaya plants are grown in semi-commercial scale. Sporadic occurrence of the disease was observed in most areas with disease incidence of less than 10%. Based on interviews, the disease was first observed to occur in these areas in the later part of 1984.

DISCUSSION

Two virus diseases of papaya, namely papaya leaf curl and papaya mosaic have been reported in the Philippines. The virus disease of papaya that is described in this paper is not related to papaya leaf curl which was previously reported by Reyes *et al.*[11]. Papaya leaf curl is characterized by severe upward curling of leaves, reduction of leafblades and petioles and it is transmitted by *B. tabaci.* On the other hand, the disease closely resembles papaya mosaic in some aspects of symptomatology[10], however, due to limited information and absence of the locally reported papaya mosaic, the relationship could not be confirmed.

Among reported virus diseases of papaya, the disease has striking similarities with papaya ringspot or distortion ringspot of papaya reported in other countries[2,3,4,5,8,12,13]. The disease produces characteristic symptoms of prominent mottling, malformation and reduction of laminae, presence of oily dark green streaks on petiole and distinct ringspots on fruit which resemble that of papaya ringspot. Host range studies, however, did not support the above observations. The results failed to demonstrate the susceptibility of some species of cucurbits to the disease as reported by Conover, de Bokx and Zettler[2,3,6,14].

Transmission tests show that the virus can be readily transmitted mechanically and it has been transmitted so far, by four species of aphids, *M. persicae* being the most efficient aphid vector. In the same manner, papaya ringspot virus (PRSV) is transmitted mechanically as well as by a number of aphids including *A. craccivora, A. gossypii* and *M. persicae*[2,5,9,14]. Although an indication of seed transmissibility of the virus has been observed, conclusive evidence has not been fully established.

Based on gross physical characterizations, the virus causing papaya disease in the Philippines fits to that of PRSV. Studies showed that it is inactivated by exposing infectious sap at 55°C for 10 minutes, by dilutions greater than 10^{-3} and after standing eight hours at room temperature. These results are in agreement with the findings of Conover[2]. Dip preparations and ultrathin sections of infected leaf tissues reveal flexous particles with particle length ranging from 780-800 nm. Ultrathin sections indicate a special band formation of particles in an almost parallel

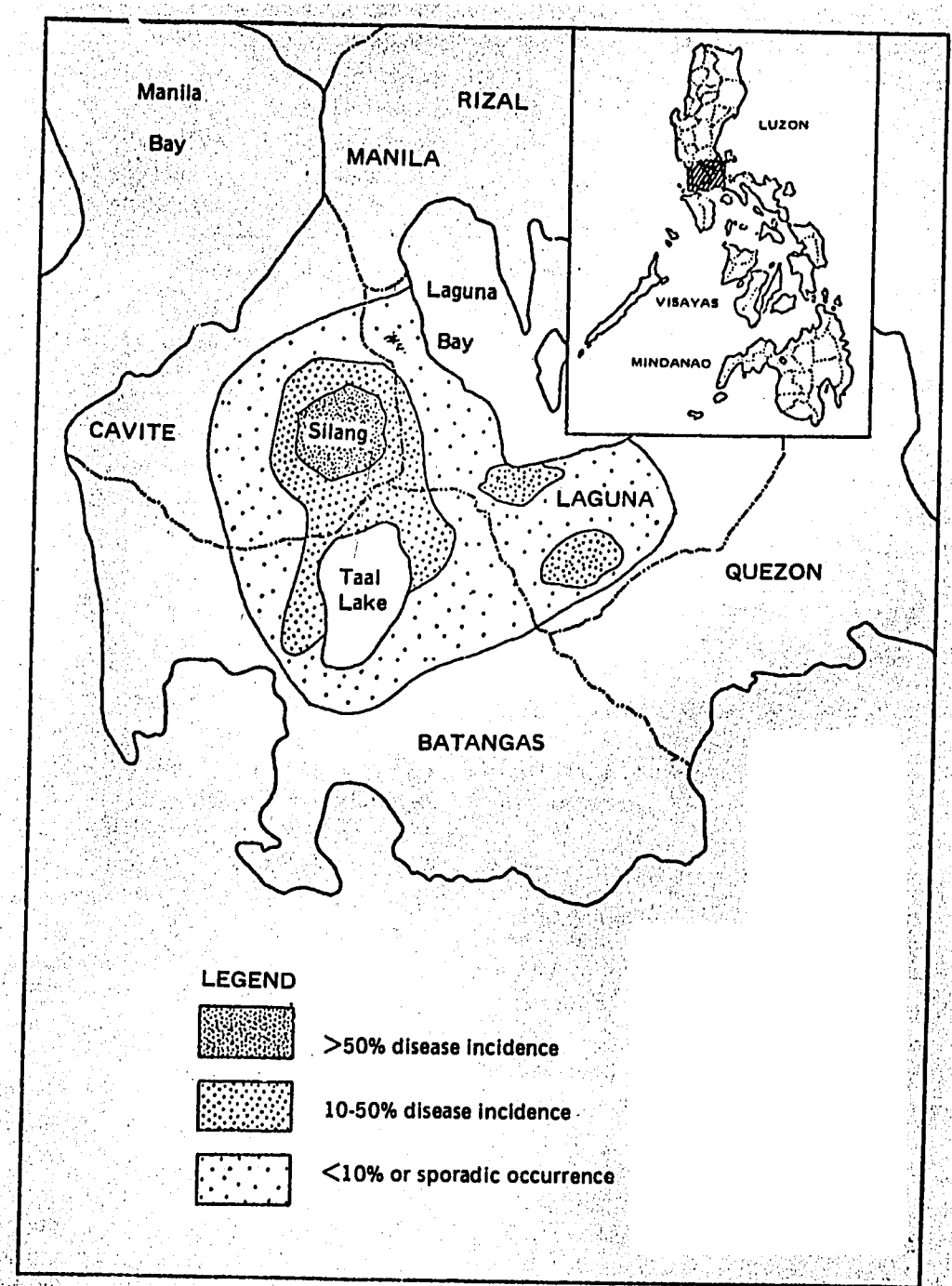

Fig. 7 Geographic distribution and incidence of the virus disease attacking papaya plants in the Philippines

arrangement which is similar to a particle formation of PRSV described by Herold and Weibel[7]. Ultrathin sections also revealed the presence of cytoplasmic inclusion bodies typical of the structures described by Zettler *et al.*[14], pinwheel and circular inclusions. Disease symptomatology and the established virus properties suggest that the virus is similar to PRSV.

Recent survey shows that the disease has occurred in three provinces and it seems to be spreading very rapidly. Although the virus is readily transmitted mechanically, rapid spread of the disease seems to indicate that the virus is primarily transmitted in the field by aphids. The occurrence of the disease in apparently isolated backyards planted to papaya suggests that the virus may be transmitted from virus sources other than infected papaya plants.

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DISCUSSION

Q. (C.N. Roistacher)
How do you think the disease came into the Philippines?

A. It is unclear. The papaya industry in the Philippines is an old industry and the disease apparantly newly introduced. Possibly it was introduced by way of Hawaii, due to its proximity, there are many Filippinos in Hawaii who possibly brought in plant material from there.

Q. (S.D. Yeh)
The varieties of papaya you grow in the Philippines, are they mostly of local varieties or introduced from Hawaii?

A. There are three important local varieties which are popular, Cavite Special, Legaspi No. 1 and Solo, but we do have some introduced from Hawaii.

Q. (F.W. Zettler)
In what form do think the virus may have come in, in seed or other?

A. We did our tests on seed transmission and we found that it is not seed transmissible, possibly diseased seedlings were introduced.

Comment: (C.N. Roistacher)
I would like again to emphasize the concept of quarantine and how important it is that you consider seriously, very strict quarantine measures. One thing that I have noticed during this conference is the sudden appearance of diseases which are initially prevalent elsewhere and they appear to crop up simultaneously around the world. The damage that some of these diseases can cause is immeasurable. I again emphasize taking home concepts of strict quarantine measures.

Comment: (O.S. Opina)
We are trying to implement such a scheme. With papaya we now have to establish the epidemology of the disease and perhaps attempt isolation of the affected areas and use state quarantine. We need to determine the isolation distance, if it is in the order of hundreds of kilometers then it will be very difficult to isolate this disease.

CONTROL OF PAPAYA RINGSPOT VIRUS BY SEEDLING INOCULATION WITH MILD VIRUS STRAINS

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SUMMARY

In the past decade, a destructive disease caused by papaya ringspot virus (PRV), a potyvirus, has become the major limiting factor for growing papaya in Taiwan. The unavailability of resistant sources and the restrictive host range of PRV make cross protection an attractive control measure. Efforts to select mild strains from natural virion population were unsuccessful. However, two mild mutants (PRV HA 5-1 and 6-1) which cause symptomless infection to papaya were induced by nitrous-acid mutation. The mutants provide a high degree of cross protection under greenhouse conditions. Results of small-plot trials and large-scale field tests indicate that the mutants are highly valuable mild strains for controlling PRV by cross protection.

摘 要

在過去之十年中，由馬鈴薯Y群病毒中之木瓜輪點病毒引起之嚴重病害已成爲臺灣栽植木瓜之最重要限制因子。因抗病之木瓜品系迄未育成，又本病毒之寄生範圍甚窄，使得利用交互保護作用之原理來防治此病害，成爲一極富潛力之策略。由自然界之木瓜輪點病毒族群中篩選輕症病毒系統，經多方努力仍未能成功。經由亞硝酸處理之人工誘變所得病毒PRV HA 5-1及6-1，在木瓜上僅造成無病徵之感染；此二突變株於温室內，證實具有高度之交互保護能力。田間之小區試驗及大規模栽培試驗之結果顯示，此二輕症病毒可作爲利用交互保護原理防治木瓜輪點病毒極具價值之疫苗。

摘 要

最近10年間potyvirusに屬するpapaya ringspot virusによる激しい病氣が台湾のパパイヤ栽培を制限する主な因子になつた。抵抗性品種がなく、また本ウイルスの寄主範圍が狭いことから交叉免疫が有効な防除法と考えられた。自然に分布するウイルスから弱毒ウイルスを選擇する試みはうまくゆかなかつた。しかし、亞硝酸處理によつて2種類の弱毒突然變異株(PRVHA 5-1と6-1)が得られ、これらはpapayaに無病徵感染する。温室條件ではこれらのmutantsに高い干渉効果をもつ。小區劃の試驗および大規模な圃場試驗の結果、これらの弱毒系統はPRVを干渉効果により良く防除することが分つた。

CONTROL OF PAPAYA RINGSPOT VIRUS BY SEEDLING INOCULATION WITH MILD VIRUS STRAINS

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INTRODUCTION

Papaya (*Carica papaya* L.) is widely grown in tropical and subtropical areas for its delicate fruit. The plant grows fast and yields fruit eight to ten months after being transplanted in the field. The delicious and nutritious fruit contains a common protease, papain, which is a digestive. The extensive adaption of this plant and wide acceptance of the fruit offer considerable promise for papaya as a commercial crop for local and export purposes. Like banana, pineapple, and mango, papaya is one of the important cash crops in the tropics and subtropics.

However, a destructive disease caused by papaya ringspot virus (PRV) is a major obstacle to wide-scale planting of this fruit tree. PRV has been reported as a major limiting factor for growing papaya in areas of Hawaii[19,23], Florida [4,7], Caribbean countries[1,19,39], South America [18,19], Africa[19,20], India[2,38], and the Far East [42]. In papaya it causes mottling and distortion of leaves, ringspots on fruit, and water-soaking streaks on stems and petioles. It stunts the plant and drastically reduces the size of the fruit[19,23]. PRV is a virus with flexuous, filamentous particles about 800 nm long. It is stylet-borne by aphids, is sap-transmissible, and has been placed in the potato virus Y (potyvirus) group[9,17]. PRV has a narrow host range which includes species of three dicotyledonous families, Caricaceae, Chenopodiaceae, and Cucurbitaceae[33]. The virus is serologically identical to watermelon mosaic virus 1 (WMV-1)[14,34,43], which is of economic importance wherever cucurbits are grown[41].

PRV has become the major limiting factor for growing papaya in Taiwan since 1975[42]. Several attempts made to develop effective control measures for PRV have been fruitless. The unavailability of PRV-resistant papaya cultivars and the restricted host range of PRV make cross protection an attractive method of controlling this virus. In the past few years, a search for an ideal mild virus strain to be used for cross protection has been conducted at Cornell University. Two mild mutants, designated as PRV HA 5-1 and PRV HA 6-1, which cause symptomless infection in papaya have been derived from nitrous acid mutagenic treatments[45]. The characteristics of these mutants, the evaluation of their cross-protection effectiveness, and their practical application as protective strains in the field are reviewed in this report.

THE PROBLEM CAUSED BY PRV IN TAIWAN

PRV was first recorded in Southern Taiwan in 1975[42]. Within two years the virus spread over the west coast of Taiwan and destroyed most

of the commercial papaya orchards there[42]. The total yield of papaya dropped from 41,595 metric tons in 1974 to 18,950 metric tons in 1977, and the wholesale price increased sixfold during the same period, from N.T.$3.67/kg (U.S.$0.04/lb) to N.T.$20.70/kg (U.S.$0.24/lb)[32]. Because of the attractiveness of the high price of the fruit, the total planting area of papaya increased, instead of decreasing, from 1,658 hectares in 1975 to 4,266 hectares in 1984. The disease devastated papaya orchards on the west coast of the island; thus the government encouraged farmers to grow papaya on the east coast isolated by high mountains; however, the virus did not take long to take hold there. Right now, Taiwan has lost its papaya export trade to Hong Kong and Japan, and the domestic supply is insufficient. Moreover, papaya tree production has become annual instead of perennial because of the severe virus infection.

The disease caused by PRV in Taiwan was first identified by Wang *et al.*[42] and further characterized by purification and electron microscopy at Chung Hsing University[21,44]. Virus strains of different pathogenicity have been reported[3,22] and their ability of cross protection has been studied[22].

CROSS PROTECTION AS A CONTROL MEASURE

Cross protection, first clarified by McKinney in 1929[24] with tobacco mosaic virus (TMV), describes the phenomenon in which plants systemically infected with one strain of a virus are protected from infection by a second related strain of the same virus. However, wide-scale adoption of cross protection for control of TMV did not occur until Rast[35] produced an almost symptomless mutant (MII-16) from a common tomato strain of TMV by the nitrous-acid mutagenic treatment [13,27]. The symptomless mutant has been manufactured commercially[36] and has been applied to a high proportion of glasshouse-grown tomato crops in the Netherlands and the United Kingdom since 1970[10,11,36]. Successful wide-scale control of tomato mosaic disease with an attenuated mutant (LII A), which was isolated from the tomato strain of TMV in plants treated with high temperature, was also reported in Japan[31]. Cross protection also has been used on a large scale to control citrus tristeza virus (CTV), a closterovirus, that is important worldwide[32]. Naturally occurring mild strains of CTV have been selected and can offer protection in the field[12,15,16,26,29,30,40]. In Brazil the number of protected sweet orange trees exceeded eight million in 1980, and no breakdown in protection has been observed[8].

Several attempts to develop effective control measures for PRV in Taiwan, such as escaping the disease by planting papaya in the season of less alate aphids, intercropping with a high-stem barrier like corn, eradication of diseased plants in orchards, spraying with mineral oil and systemic insecticides, and protecting young seedlings with plastic bags after transplanting have proved either ineffective or only of marginal benefit. Although tolerant selections of papaya have been described[5,6], resistance to PRV does not occur within *C. papaya* [5,6,7,42]. Some species of *Carica* are resistant to PRV[5,6,7,25] but unfortunately these species are incompatible with *C. papaya* and conventional interspecific hybridization has been unsuccessful [25]. A diligent rogueing program has been practiced successfully in Hawaii to suppress the spread of PRV in certain areas of the state[28]. However, rogueing is not a permanent solution for an area without geographic isolation, and it is impossible to eradicate the virus sources in a place like Taiwan where the disease has become epidemic.

The severe crop losses, the unavailability of PRV-resistant papaya varieties, the difficulty of eradication, and the restrictive host range of PRV make cross protection an attractive method of controlling this virus.

SEARCH FOR MILD VIRUS STRAINS FROM NATURAL COLLECTION

The key for practical application of cross protection is that there must be a useful protective virus strain. One mild strain, PRV Su-mm, which was selected from 230 PRV isolates in

Taiwan[22], offered a certain degree of protection to papaya against infection by severe strains[22]. Unfortunately, this naturally occurring strain was not mild enough and can not be used for cross protection[22]. Attempts to select mild strains from 116 isolates collected from Hawaii were also unsuccessful[45]. Although some of the natural variants by single-lesion isolation from natural populations caused various degrees of symptom severity on papaya seedlings, none proved a useful strain[45]. As these efforts proved fruitless, it seems that the mild strains are not present, or at least difficult to isolate, in nature. This is different from citrus tristeza virus for which mild strains are easily collected in the field and are used for the practical application of cross protection[8,26].

SYMPTOMLESS MUTANTS FROM MUTAGENIC TREATMENTS

Since efforts to select naturally occurring mild strains of PRV by field collection, or single-lesion isolation from natural virion populations were not successful, the emphasis was shifted to artificial mutagenesis. Nitrous acid, a powerful chemical mutagen for plant RNA viruses[13,27,37], was used to induce mutants from PRV HA, a severe strain of PRV isolated in Hawaii[14]. Crude sap from PRV-infected squash was treated with nitrous acid (pH6.0) at 20°C for 30 minutes and used to inoculate *Chenopodium quinoa*. Single local lesions on *C. quinoa* were transferred to papaya seedlings 20 to 30 days later. Two mutants, designated as PRV HA 5-1 and PRV HA 6-1, that produced no symptoms on papaya, were obtained from 663 single-lesion isolations in July 1982[45]. Papaya seedlings inoculated with these two isolates remained symptomless or showed diffuse mottling with no reduction in plant size. Seedlings of *Cucumis metuliferus* and Zucchini squash infected with these isolates exhibited light vein-clearing with no reduction in vigor or growth. All the plants infected with PRV HA 5-1 or PRV HA 6-1 had strong positive reactions when tested with ELISA after inoculation. This indicated that symptomless infection was not due to low concentration or slow multiplication of the virus.

The mutants satisfy the first requirement to be an ideal mild strain that does not cause severe damage to the crop protected. Moreover, the mutants not only cause symptomless infection to the most susceptible cucurbitaceous plants, *C. metuliferus* and Zucchini squash[45], but also behave similarly in melons, watermelons and cucumbers. Because the systemic hosts of PRV are limited only to Caricaceae and Cucurbitaceae and the mutants cause symptomless infection to the plants in these two families, the possibility of damaging the cucurbitaceous crops in the vicinity of papaya orchards which are protected with mild mutants is minimal.

MILD MUTANTS CROSS PROTECTION EFFECTIVENESS

Under Greenhouse Conditions

In order to test the cross protection effectiveness of the symptomless mutants, PRV HA 5-1 was subsequently used to protect papaya plants against challenge inoculation with a severe HA strain of PRV under greenhouse conditions[45]. The tests were conducted at Cornell University from October 1982 to April 1983. When papaya plants were protected with PRV HA 5-1 and then challenged with a severe HA strain at different time intervals, a high proportion (79-93%) of the plants remained symptomless even 60 or 90 days after challenge inoculation when the time interval between preinoculation and challenge inoculation was increased to 26, 35 and 56 days. Results of cross protection between PRV HA 5-1 and PRV HA after challenge inoculation to different leaf positions were also investigated. Papaya seedlings inoculated with buffer first and reinoculated with HA showed severe symptoms 15-25 days later. In all plants challenged at different leaf positions, severe symptoms were not observed for at least 30 days in 80% of the plants. A high proportion (90%) of plants that were challenge inoculated on expanded leaves remained protected 90 days after challenge inoculation. However, a majority (50-80%) of the plants that were challenge inoculated on the upper nonexpanded young leaves, or all

leaves developed symptoms 60-90 days after challenge inoculation. This indicated that the upper young leaves around the apex were weak points for the invasion of the severe strain.

When the plants preinfected with PRV HA 5-1 were continually challenged at different leaf positions, 30, 32, 34 and 36 days after initial inoculation, nearly all plants remained symptomless 90 days after the first challenge inoculation.

In general, either complete or a high degree of protection was observed when PRV HA 5-1 was used to protect papaya against the severe effects of infection by the parent strain HA under various mechanical challenge treatments. PRV HA 6-1, another symptomless mutant, also offers similar capability of cross protection against HA severe strain and two Taiwanese severe strains. The results indicate a good potential for the use of the mutants as protectants for the control of PRV.

Under Field Conditions

In the spring of 1983, the symptomless mutant HA 5-1 was tested in Hawaii to evaluate its capability of cross protection under field conditions. The papaya plants inoculated with HA 5-1 exhibited normal growth and fruit-setting the same as healthy plants. The horticultural properties of papaya were not affected by the mild mutant. This preliminary test was mainly for the observation of the possible damage by the mild strain. Although a high degree of protection and tremendous increase in yield were observed in the protected plants, the data were not carefully analyzed because the small number of plants used was not statistically meaningful. Large-scale tests were conducted in Hawaii in 1984, but the severe drought ruined all the test plants. A new field trial was established in the spring of 1985.

A total of 730 papaya plants protected with HA 6-1 and 730 unprotected controls were tested in three severely diseased areas in Taiwan under natural conditions in the fall of 1983. Mass inoculation was achieved by pressure spray (8 kg/cm^2, at 10-20 cm distance), using inoculum prepared from *C. metuliferus* in 0.01 M phosphate buffer (pH 7.0, 10 ml/g) and mixed with carborundum. In Feng-Shan and Kao-Hsu areas, where the protected papaya were mixed with the unprotected control at random, or row by row under high challenge pressure, unprotected plants showed severe symptoms two to three months after planting, and, the protected plants held out only one to two months longer than the controls, with no economic benefit because the breakdown occured before fruitset. However, in a solid-block test at Ta-Liao, where the challenge pressure inside the test orchard was minimized by rogueing, protected trees showed 80% increase in yield compared to the controls. Moreover, the total income increased 110% because of better fruit quality.

The differences among Feng-Shan, Kao-Hsu, and Ta-Liao might be due to the differences in challenge pressure and planting designs. After the healthy plants, which were mix planted with protected ones, became infected with a severe virus they provided too great a challenge pressure to the protected ones surrounding them. This may explain why the benefit of cross protection only lasted for one to two months in the Feng-Shan and Kao-Hsu areas. Since, in the real situation, no growers would grow their protected papaya plants mixed with unprotected ones, thus, the solid-block test at Ta-Liao is more similar to actual orchard conditions, and its success brought hope for use of cross protection to control the devastating PRV in Taiwan.

The government proceeded with large-scale planting in the spring and fall of 1984 with 44,000 protected plants (22 hectares) and 200,000 protected plants (100 hectares) in the field, respectively. Up to the end of 1984, the average disease incidence of protected orchards from the spring planting was 31.1%, compared to 82% of that of unprotected controls. The average fruit yield per tree increased from 7.3 kg for unprotected trees to 17.9 kg for protected ones. The income of the growers from protected fields was 109% more than that of unprotected ones. Results of the fall, 1984, planting are not complete. At the end of June 1985, the severe disease incidence of

protected orchards was 19.40%, compared to 45-100% of unprotected controls.

Thus, the preliminary data of large-scale trials, using the symptomless mutant as a protective strain, indicates a very significant reduction of severe disease incidence and a tremendous increase in the fruit yield of papaya.

CONCLUSION

Although cross protection is a general phenomenon of plant viruses, not all plant diseases caused by viruses can be controlled by using a mild virus strain for preimmunization. Criteria for practical application of cross protection are that the mild virus strain:

1) Does not cause severe damage to the protected plants.
2) Must be stable for a long period.
3) Protects plants against the effects of severe strains.
4) Must be suitable for infecting plants.
5) Does not affect other crops in the vicinity of the protected crop.
6) Has no synergistic reactions with other viruses.

The symptomless mutants of PRV, HA 5-1 and 6-1, satisfy all the above criteria. Evaluation of their cross protection effectiveness, both in the greenhouse and in the field, proves that the symptomless mutants are highly valuable mild virus strains for control of PRV by cross protection.

In certain conditions of greenhouse tests and field trials, when the challenge virus was imposed on the nonexpanded young leaves around the apex or the protected plants were surrounded by a severe challenge pressure, the effect of cross protection was observed only in the delay of expression of severe symptoms. If the breakdown of cross protection happened before flowering, there would be no economic benefit. More studies are needed to monitor the mild mutants in the field, to compare their capability against different severe strains, and to correlate the breakdowns to the population density of alate aphids and the inoculum density of severe strains. These efforts will help minimize the incidence of breakdown.

At present, rogueing of diseased plants in the protected orchard, protecting seedlings with a plastic bag after transplanting, and intercropping with a high stem barrier like corn are recommended to reduce the challenge pressure. The agricultural practice of supplementation with suitable fertilizers to enhance the vigor of protected trees is also recommended. Recently, a tolerant variety introduced from Florida (kindly donated by Dr. R.A. Conover) has been released in the field. The tolerant variety protected with HA 5-1 significantly increases the yield and quality of the fruit. The integration of cross protection with agricultural control measures and the tolerant variety of papaya brings hope to restore the normal production of papaya in Taiwan.

After success in the fall planting of 1984, the Council of Agriculture of the Republic of China plans to expand the protected orchards up to 220 hectares in the fall of 1985. More than 610,000 papaya seedlings will be preinoculated with PRV HA 5-1 or 6-1 and then released to the field. Using the induced mild virus mutants to preimmunize papaya seedlings for control of PRV may become a routine practice in Taiwan. This will be the world's first case of a successful large-scale application of cross protection to control an aphid-nonpersistently-transmitted potyvirus.

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DISCUSSION

Q. (O.S. Opina)
At present the strain used does not cause any damage to cucurbits, but with genetic variation introduced through the breeding program isn't it possible that sooner or later this could happen?

A. Yes! Therefore we have to check all strains to see the effect of their variation, so far those we are using are safe to watermelons, Chinese melons and cucumbers. We cannot avoid this challenge with new varieties.

Q. Some countries are very strict on quarantine. Have you ever imported infected fruit in the course of your work, and what are the implications of this? How does a country like Taiwan now export fruit?

A. So far we have not considered this problem of export, I expect there would now be a problem exporting to Japan.

Q. (F.W. Zettler)
What is the rate of transmission of the mild strain compared with that of the severe strain? In the field will the mild strain outstrip the severe strain simply by faster transmission?

A. We have seen that the protected trees planted beside healthy controls can be a source of mild strain infection for those controls, and we assume this transmission is by the aphid vector. We can only hope that the mild strain by this means may become dominant.

Q. (C.N. Roistacher)
With the tristeza cross protection that we have been doing, we have found that because of the closterovirus being such a large gene, there is breakdown. Strains occur that breakdown the effect of our mild protection. And so I would suggest that you must continue creating new mild strains and you should not be disappointed if there is gradual breakdown because of the development of new strains. Especially, if you have a surrounding host in which these strains can evolve and develop. Hopefully this will not happen, and I am sure you are planning to develop more mild strains.

A. Yes! This strain is from Hawaii and not of our own severe strain in origin, in which case the relationship between the severe and mild strains would be closer and the protection better. As to the breakdown in the field we are not really sure of the reasons, and here it may be in part that the relationship between the two strains is not so close. So what we are trying to do is to produce our own mutant from the local severe strain. This we have worked on for several months.

Q. (M. Koizumi)
You now have two mild strains are there any differences in their activity? You have different severe strains, when you protect against these are there differences in response?

A. Yes! There are some differences, first strain 5H 1 has a higher degree of protection than strain 6H 1. If we challenge the protected trees with different severe strains, the mosaic strain or the wilt strain

(we have mostly the mosaic type) we see a difference. The breakdown is more when the wilt strain is used as challenge than with the mosaic strain. We have many severe strains in nature and cannot expect that our mild strain can protect against all of them. We do not expect the protected trees to be 100% immune but only can expect to increase the production and income of the farmer which is most important. We still have a lot of problems.

SECTION IV

GENERAL DISCUSSION

GENERAL DISCUSSION

Comment (R.J. Chiu)

During the last two and a half days more than twenty papers have been presented discussing virus and virus-like diseases of different fruit and vegetable crops. Some of these diseases, particularly those due to geminiviruses appear to be new to many countries or regions but many are common and worldwide in distribution. So we have citrus tristeza virus distributed everywhere that citrus is grown, greening disease appears to be another common disease problem in most Asian countries though under different names, and, we have cucumber mosaic virus, watermelon mosaic virus 1, and potato virus Y which are common problems for a number of vegetables which are grown worldwide. To plant virologists these disease problems present a great challenge, and an unusual research opportunity. The control of virus disease, the winning of this battle depends very much on an intergrated series of disease control measures under a sustained effort. At this seminar I am delighted to hear of the many advances in these control programs. Sources of virus resistance are now available for several important viruses in vegetables and also in a few fruit crops. The protection of plants with mild virus strains against severe ones is now not just a theoretical concept but has already greatly benefited growers who have adopted this in control programs. The importance of establishing a disease free plant propagation scheme in combating fruit free viruses needs emphasis. In this respect some countries have accomplished very much, while others are just beginning, making slow but steady progress. In the fields of virology and electron microscopy new techniques are continually being developed, these advances are making virus identification not only possible but easier and more reliable.

F.W. Zettler

The most controversial issue raised in this symposium is that of cross protection, and I would first like to pose the question as to the pros and cons of this approach. Has the issue of eradication been answered thoroughly enough? I believe in the island of Molokai that papaya ringspot is extremely severe, but I believe it was introduced to the island of Hawaii but then eradicated. Would you like to comment on that Dr. Gonsalves?

D. Gonsalves

The virus was first found on the island of Molokai, and then subsequently spread to the island of Hawaii, where something like 80% of Hawaii's papaya are grown. In reality Hawaii has done a remarkable job of keeping the virus out of the main growing area which is essentially the area of Kuna where there is a monoculture of papaya, and where 70% of Hawaii's papaya are produced. To this day the virus is not in that locality, though it is in surrounding localities, in the districts of Hilo and the district of Corna, in miles about 40 to 50 miles away. Fortunately we have a very effective geographical isolation. The people of Hawaii realized that if the virus gets into Kuna we would be in really bad shape. So in the island of Hawaii alone we have five government workers whose only job is to go around daily in the districts of Hilo and Corna and Kuna looking for any plants with symptoms of ringspot. If they see any plant with symptoms they have the right to go into private yards and chop them down. Using this method it is suprising that they have essentially limited the spread. As an example, a few years back the virus was identified in a village quite close to the Kuna district. Personnel from the State Department went into the village and chopped down every papaya tree, and pulled out every native host plant in that village and they simply eradicated the virus. So in Hawaii they are using this basic eradication procedure to prevent the virus getting into the growing areas. And very fortunately, and to me it is suprising, they have been able to do this.

F.W. Zettler

What would you recommend in the situation in the Philippines where the virus has recently been introduced? Do you recommend the cross protection approach or the eradication approach?

D. Gonsalves

My approach is quite similar to the one I recommended in Hawaii. The reason that I started my cross protection work was that in 1978 the dean of the University of Hawaii asked me what was the potential of the Kuna district becoming infected. My response was that there was this possibility and that instead of waiting and reacting to the problem we should anticipate it and develop preventative measures in case the virus did get away. This would be my same recommendation for the Philippines. But it is my understanding from where the virus is already, that it has probably spread beyond any reasonable attempt for eradication; however, in isolated parts of the Philippines where the virus is not yet established, I would recommend that they take very strict quarantine measures.

Then the question is; do you use cross protection in the areas that are already infected? I think that in the different areas where they find the virus very widespread then the subsequent crop will be affected and cross protection will be necessary. Then the question becomes one of making a balance.

With cooperation one approach may be to try cutting down as many of the infected plants as possible and planting nothing but the protected plants. May be this will overcome the population of the severe strain, then crop protection would be even more effective. But the important thing is that you limit the multiplication of the severe strain, and eventually you may be able to get away from cross protection because you have effectively eliminated the severe strain. This may be entirely theoretical but it is well worth investigating.

Observer

In the case of the ringspot virus in Taiwan, it is understood to have a very limited host range, and the virus is not seed transmissible. Furthermore, because of the ringspot disease the papaya plantations in Taiwan have now become annual or biannual. Taiwan is a small island and geographical isolation of some areas should be possible due to the mountain range. Given these conditions before we have a strong resistant variety that can be released, and, before we have a very good cross protection method, is it possible that we could eradicate all the virus effected papaya in one season, and eliminate all the inoculum in this island?

R.J. Chiu

The first time papaya ringspot was found in Taiwan was 1975. At that time most of the papaya planting area was in the west of the island with very little in the east. The central mountain range separates the island into an eastern and western part. In 1977 when the virus disease was getting serious we tried to move the papaya cultivation from the western part to the eastern part. The government undertook a project to distribute as many as 340,000 seedlings, distributed free to growers on the eastern part. But we were not as efficient as the growers of seedlings, and businessmen, they shipped seedlings from the western part to the east. We were unsuccessful in preventing the virus spread. Had we been successful then I think we would not have had to go to cross protection. Now our island is so small that we cannot find a part free of the virus. I do not think it is possible for us now to clean out any area so that we can grow virus free papaya. The spread of

papaya ringspot was so quick that even isolated trees in the cities on the street sides are infected with the disease, though they are well separated from the growing areas.

S.D. Yeh

It would not only be necessary to remove all the trees in order to remove all the sources of inoculum, but also there are the other natural hosts, which are already known to harbor the virus. The virus is everywhere, not only in papaya but also in the cucurbits, so I would say that is impossible to remove all these plants.

F.W. Zettler

I have a little familarity with papaya in Florida. Florida in a sense is also very small as Taiwan. I don't think that small size necessarily precludes isolation. The reason I say that is due to a study done under Dr. Thomphson, this survey was published in Plant Disease in about 1982. They did a very careful survey of the papaya in the eastern part of Florida and in the western part of Florida. Florida does not have a mountain range but there is a plant barrier between the east and the west. They found a very high incidence in proportion to commercial production in the east but they did not find any incidence of the virus on the west coast. I don't disagree with what you say about native plants and cucurbits being alternative sources of inoculum, but I believe personally that if papaya ringspot could become readily established in indigenous plants then it would have easily established itself along the west coast. Papaya is not raised on the western coast because it gets frosts which preclude papaya production although there are backyard papayas. I think isolation is possible at least under the Florida conditions and possibly in Taiwan.

R.J. Chiu

In the early days when we had tried to prevent the disease in a certain area on the east coast of Taiwan, our government workers tried to persuade the growers to cut down the infected trees. However, the growers responded that if they cut down the trees, the growers would cut off their heads. I think that may happen in any location.

M. Iwaki

When you tried to eradicate the trees in Florida did you pay any compensation to the growers.

D. Gonsalves

Yes! When we went to the small towns to eradicate they did not pay any money, but they offered free papaya seeds. However, there were incidences when they litterally had to cut the papaya trees and run with irate people chasing them.

C.N. Roistacher

In central California we have a tristeza eradication program. Inadvertantly a grower brought in a few thousand trees infected with tristeza, into this one remaining area which we considered tristeza free. A program was developed to eradicate these trees. When we got into this program with tests on many Mexican limes it was surprising to us. We found in addition to the few thousand trees that were brought in, that many people had broken the quarantine regulations and in fact tristeza was widespread in unbelieveable proportions. But fortunately tristeza does not spread as rapidly as in other areas in the valley concerned because of the warm climate, and with the advent of the licensing system the eradication program has been effective, but it has been difficult. We now have it under control.

With regard to the ringspot virus I would like to ask, if the mild strain becomes endemic, what is the feeling of those trained in this field as to whether it will multiply in the crop but actually the severe strain will still continue to exist within the wild hosts?

D. Gonsalves

With regard to the effect of alternate hosts for papaya ringspot. I think definitely the papaya ringspot is there, but I think that relatively speaking it is probably a minor aspect of the epidemiology of the virus. This is for several reasons, one being that it is not too serious a virus to cucurbits. Likewise the watermelon mosaic virus 1 complex is virtually identical to PRV except that it does not go into papaya. Perhaps it dominates the cucurbits and effectively crowds out the papaya ringspot. This may be one reason why the cucurbits are not a very effective host for the papaya ringspot.

F.W. Zettler

If that were the case then with the watermelon mosaic virus 1 in Florida, why then don't you find papaya ringspot virus in the watermelon of Florida? The actual incidence of virus occurrence except on papaya plantations is rather low.

Also WMV 1 is not ubiquitously established throughout all the weeds. There are some viruses in Florida that are able to survive year after year because there is an annual transition from one weed to the next or the weeds are perennial, except in the extreme south of Florida where you don't have frequent crops. That not being the case here, my understanding is that watermelon mosaic virus 1 affects only watermelon.

Papaya ringspot does not, but since papaya particularly on the east coast are so much affected by papaya ringspot, why don't we have papaya ringspot in watermelon as well?

D. Gonsalves

From my observation of papaya ringspot if compared to watermelon mosaic virus 1, I think a primary difference is that watermelon mosaic virus 1 was in Taiwan, Hawaii and Florida long before papaya ringspot ever came in. My feeling is the watermelon mosaic virus 1 effectively cross protects the cucurbits against the establishment of papaya ringspot virus.

F.W. Zettler

I wonder if in Taiwan we should not recommend a dual approach. The cross protection approach is expensive and it must be sustained over a long period of time, likewise the Hawaiian situation of eradication is also expensive, if not downright dangerous. Possibly in Taiwan, some parts could attempt an eradication program while other parts should attempt a cross protection program.

C.W. Gao

In Taiwan now cross protection seems to be the only form of control for papaya ringspot disease. I would like to ask a question about the program. Dr. Yeh has inoculated the mild strain to the papaya seedlings. Is there any possibility of inoculating this mild strain to the plant again once planted in the field? Is there any technical problem in this approach?

S.D. Yeh

I think that technically it would be possible but the cost may be high. Inoculating the seedlings, one

can inoculate thousands of seedlings at one time. So the cost is low. Once planted in the field reinoculation would be expensive. Another point is that after inoculation as a seedling, once we have a positive result to the mild strain causing systemic infection, the virus is already in the plant. Though the expression of the infection may vary under different conditions, however, it should remain in the tree for the life of the plant and I do not see the necessity of reinoculation in the field.

C.N. Roistacher

I was trying to find out if we had multiplication of the virus in the weed hosts and did not really get an answer to this. I feel that we should make an assumption and temper our optimism. Sooner or later severe strains will super impose on our mild strains, and I think that has already been shown in two of your plots in Taiwan where you were surrounded by various other sources of inoculum. There is a potential of overriding this mild strain. Thus I urge the funding of continued search for additional mild strains, and the development of a bank of mild strains against the event of eventual breakdown of control. I would also like to comment on the very excellent work of those who have developed these mild strains, it is very systematic and fine work, and should be commended.

O.S. Opina

As I mentioned in the Philippines we have call to develop a research program, with the aim of developing an intergrated management scheme for tackling this papaya ringspot disease, because it seems to me that the individual control tactics when applied individually are not enough. Probably the best solution for the Philippines will be to intergrate all these control tactics in an overall scheme. That should be the ultimate aim of our research program. I wonder if in Taiwan, have you developed an intergrated management scheme, or does one exist in Florida?

D.Gonsalves

When deciding where and when to test this mild strain, the aim of these experiments in Taiwan was to try to figure out how to use the mild strain. I really believe that one should not look only at cross protection but at it as a part of an intergrated pest management program. Everybody uses pest management for insects, but I think this process of learning how to use cross protection is a real example of intergrated pest management. We have already shown that for example if you plant in the spring in Taiwan you get good initial crop growth. But then the crop comes into bearing just at the time of lower fruit quality due to the season and there is poor demand. Though theoretically it seems nice, but practically it is not a fesible method. So we even have to think of time of planting.

Definitely I would never try to use cross protection in a field that is next to one 100% infected, I think it has to be done in isolation. I definitely agree with you that cross protection is just one of the ways of control and if a tolerant variety, an acceptable commercial tolerant variety becomes available, and I think it will become available quite soon then cross protection and tolerance would be a more ideal combination.

F.W. Zettler

In conclusion I would like to remind all that man is the origin of our present virus problems, the initiator of this man *vs* virus battle.

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