DIAPHORINA CITRI KUWAY., A VECTOR OF THE GREENING DISEASE OF CITRUS IN INDIA

S. P. CAPOOR, D. G. RAO and S. M. VISWANATH

Indian Agricultural Research Institute, Plant Virus Research Laboratory, Poona

Received : April 17, 1967

McClean and Oberholzer (1965a) described the greening disease of citrus from South Africa and produced evidence to show that it is caused by a transmissible virus. A similar disease affecting sweet-orange (*mosambi*) was observed by the senior author in Andhra Pradesh in 1960 and was transmitted by grafting to greenhouse raised healthy seedlings of sweet-orange [*Citrus sinensis* (L.) Osbeck], Rangpur lime (limao cravo; *C. limonia* Osbeck), Indian acid-lime [*kagzi nimbu*; *C. aurantifolia* (Christm.) Swingle], and grapefruit (*C. paradisi* Macf.) cultivar ' Marsh Seedless ' at Poona (Capoor, 1966). The identity of the disease and its similarity to the greening disease of South Africa was confirmed in 1966 (Fraser and Singh, 1966; Fraser *et al.*, 1966). Schneider (1966) observed a necrosis of sieve tubes in leaves and in fruits of sweet-orange affected by the greening virus. Large amounts of phloem formed in the leaf veins as a result of the necrosis and caused the veins to be enlarged.

The studies reported in the present paper deal with the transmission of the greening virus by the adults of *Diaphorina citri* Kuway., the species of psyllid commonly found on citrus in the country. The insect transmission tests were conducted during June–November 1966, when the population of citrus psylla is usually high on citrus trees in the central and southern India. *Diaphorina citri* is yet another species of psyllid responsible for the spread of the greening virus (Capoor *et al.*, 1966), since the South African greening virus is spread by a psylla, *Trioza* (*Spanioza*) *erytreae* del Guercio (McClean and Oberholzer, 1965b).

MATERIAL AND METHODS

The psylla were collected from diseased trees of sweet-orange in the Ganeshkhind Fruit Experiment Station, Kirkee, and transferred to young seedlings of sweet-orange (*mosambi*), grapefruit (' Marsh Seedless '), and acid-lime which had been grown from seed inside glass-house and potted singly in 20-cm earthen pots. Each test seedling was enclosed in a lantern glass globe, the upper end of which was covered with voile cloth fixed with a rubber band. The adult psylla were released inside the globe and the pots kept in the dark to make the psyllids settle down to feed upon the test plants as soon after their release inside the globe as possible. The psylla were liberated in lots of 40 to 95 adults per test seedling which was exposed to their feeding for 12 to 15 days (Table I, Series I). At the end of feeding exposure the plants were sprayed with 0·02 per cent Ekatin emulsion to kill the insects and kept in a glass-house under observation. Thereafter the plants were sprayed with Ekatin at intervals of 15 days in order to prevent re-infestation of the test plants by the psyllid.

A colony of virus-free psyllids was raised on healthy seedlings of sweet-orange and maintained on them in the insectary. Insects from this colony were used in specific transmission tests only. For many transmission tests carried out later the adult psylla were usually collected from any citrus species on which good colonies were observed. The insects bred and colonized mostly on the young developing shoots and flower buds. They were largely collected from *Citropsis schweinfurthi* (Engl.) Swingle and Kellerman, on which they were abundant in the premises of Plant Virus Laboratory, Poona, for a long period in the year.

The psyllids collected from sweet-orange, grapefruit, mandarin and lime in other orchards were fed for 24 hours on selected source twigs respectively of sweet-orange, grapefruit, mandarin, or lime cut from diseased trees and kept in water. The feeding for virus acquisition as well as transmission was carried out in the dark chamber. All source twigs collected from diseased trees in orchards carried both the greening and the tristeza viruses. In these tests (Table I, Series II, III and IV), the psyllids were liberated in lots of 50 to 150 and allowed to feed on test seedlings for 12 to 22 days.

RESULTS

The test seedlings were defoliated except for four or five youngest leaves before the psyllids were released on them for transmission feeding (Fig. 1, *A*). In these experiments the virus was transmitted successfully in most of the test seedlings exposed to psylla. The results are given in Table I. Similar tests carried out with nymphs have not yet shown transmission of greening in any of the 12 sweet-orange seedlings exposed to their feeding. All the untreated seedlings of sweet-orange, grapefruit, mandarin (*G. reticulata* Blanco), and lime kept as controls are still normal. Also, healthy seedlings of sweet-orange and lime were exposed to the feeding of virus-free

TABLE I. TRANSMISSION OF THE GREENING DISEASE OF CITRUS BY THE ADULTS OF *DIAPHORINA CITRI* KUWAY.

Series	Source of greening virus	number of insects (per seedling)	Period fed on test seedlings (days)	Test seedlings	Number of seedlings exposed	Number positive for greening
I	Sweet orange	50– 95	12–14	Sweet orange	12	10
	Sweet orange	85	15	Grapefruit	10	6
	Sweet orange	40– 60	15	Acid lime	6	5
II	Grapefruit	55– 90	14	Sweet orange	6	6
	Grapefruit	100	18–22	Grapefruit	8	6
III	Mandarin	55	15	Sweet orange	8	5
	Mandarin	50	12	Mandarin	8	4
IV	Acid lime	120–150	15	Mandarin	5	2
	Acid lime	120–150	15	Acid lime	6	4

psyllids in cages and kept as controls for the duration of the experiments after spraying with Ekatin. None of these developed symptoms of greening.

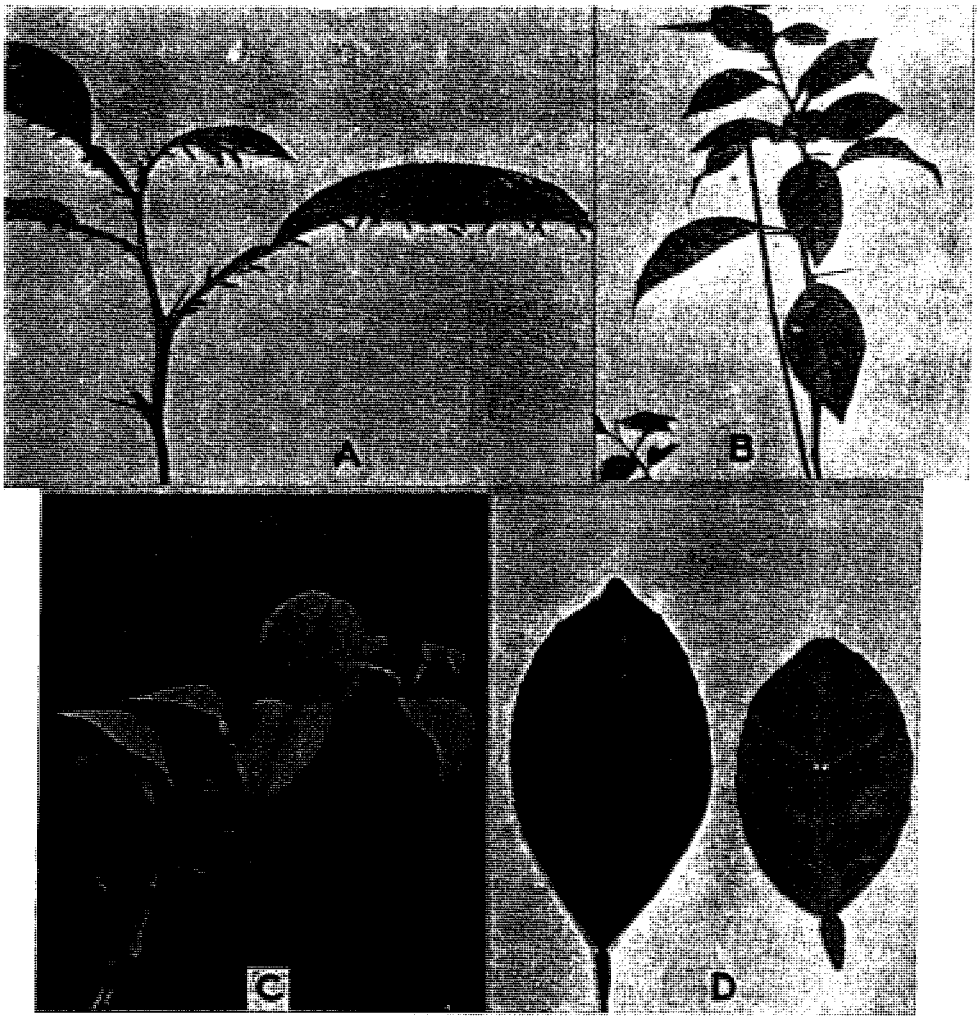

FIG. 1. SWEET-ORANGE TEST SEEDLINGS INFECTED BY THE GREENING VIRUS WHEN EXPOSED TO *Diaphorina citri* KUWAY. (*A*) SWEET-ORANGE SEEDLING EXPOSED TO ADULT PSYLLIDS. (*B*) FIRST SYMPTOMS OF GREENING AS YELLOW PATCH ON OLDER LEAF OF SWEET-ORANGE SEEDLING. (*C*) SYMPTOMS OF YELLOWING OF VEINS OF LEAF AND COMPLETE YELLOWING OF TERMINAL LEAVES OF SWEET-ORANGE. (*D*) LEAF OF INFECTED SWEET-ORANGE, SHOWING YELLOWING OF MID-RIB AND LATERAL VEINS; HEALTHY LEAF ON LEFT.

The results of these experiments (Table I, Series 1) show that the greening virus was transmitted in 10 out of 12 sweet-orange seedlings, in 6 out of 10 grapefruit seedlings, and in 5 out of 6 lime seedlings exposed to adult psylla which had acquired the virus from diseased sweet-orange in orchard. Further, the insects were also able

to acquire the virus from diseased grapefruit, mandarin, and lime, and transmit it successfully to test seedlings of sweet-orange, grapefruit mandarin, and lime (Table I, Series II, III, and IV).

The early symptoms in sweet-orange are the appearance of bright-yellow patches on older leaves (Fig. 1, *B*), followed by the yellowing of veins of younger leaves and complete yellowing of the terminal leaves (Fig. 1, *C*). These symptoms were similar to those induced in sweet-orange seedlings by grafting from affected orchard trees. Yellowing of mid-rib and lateral veins of leaves of sweet-orange (Fig. 1, *D*) appears in 70 days or after. In grapefruit the early symptom is a bright-yellow spot or spots on leaves. Other symptoms appear about 70 days after exposure to the insects. In lime the symptoms are yellowing of veins and chlorotic spotting of younger leaves, severe stunting of new shoots, and yellowing of the margins of subsequently developing leaves The early symptoms in mandarin appear after 20 days and consist of vein-clearing of the second or third leaf, followed by chlorotic spotting in subsequent leaves.

Diaphorina citri did not transmit the tristeza virus in any of the test seedlings on which they fed. This was further confirmed by back-indexing the greened sweet-orange, grapefruit, and lime seedlings on to lime test plants (Capoor, 1961), both by grafting and through the citrus aphid (*Toxoptera citricida* Kirk.). In no case the tristeza virus infection was obtained in lime plants.

SUMMARY AND CONCLUSIONS

The citrus psylla, *Diaphorina citri* Kuway., has been shown to transmit positively the greening virus to healthy seedlings of sweet-orange [*mosambi*; *Citrus sinensis* (L.) Osbeck], grapefruit (*C. paradisi* Macf.) cultivar ' Marsh Seedless ', mandarin (*C. reticulata* Blanco), and lime Indian acid lime ; *kagzi nimbu*; *C. aurantifolia* (Christm.) Swingle] from diseased trees of sweet-orange (*mosambi*), grapefruit ' Marsh Seedless ', mandarin and lime in orchard. The early symptoms are localized yellow spots on leaves of sweet-orange and grapefruit followed by general yellowing of terminal leaves and yellowing of veins in the subsequent leaves, chlorotic spotting of young leaves, followed by severe stunting and marginal yellowing of growing leaves of lime, and vein-yellowing in leaves of mandarin. The initial symptoms of greening appeared in 20 to 45 days after the psyllid had fed on test seedlings. None of the untreated control plants kept in the greenhouse developed symptoms of greening; nor healthy seedlings of sweet-orange and lime fed upon by virus-free psyllids developed any symptoms of greening. The nymphs of psylla did not transmit the virus in the tests carried out so far.

This is the first report of transmissin of the greening virus by *Diaphorina citri* Kuway., from India. *D. citri* is the second species of psyllid shown to transmit greening, the first being *Trioza erytreae* del Guercio, reported from South Africa (McClean and Oberholzer, 1965b), and the third species known to transmit a plant virus disease. Earlier, Jensen *et al.* (1964) reported transmission of pear decline virus by the pear psylla, *Psylla pyricola* Foerster.

ACKNOWLEDGEMENT

The authors are thankful to Dr S. P. Raychaudhuri, the Head of the Division of Mycology and Plant Pathology, New Delhi, for facilities.

of its release, was the first variety in the world to possess simultaneously a high degree of resistance to black, brown and yellow rusts.

After 1947, when the Fertilizer Factory at Sindhri was established, the need for the breeding of varieties capable of responding well to fertilizer application was recognized. One of the varieties developed under this programme of breeding for high-fertility conditions was NP 824. This variety is still very popular in Gujarat. The chapter on wheat breeding refers in detail to the work done at the I.A.R.I. as well as in the different states of India. A complete list of the important wheat varieties is given and the improved varieties of *durum* and *dicoccum* are also described.

Usually, the emphasis in crop improvement work tends to be placed only on yield. It is therefore a welcome feature of Dr Pal's book that there is an entire chapter on grain quality which refers to some of the unique properties of the wheat protein. Again, it is not only the quantity of protein that is important but also the quality, as studied through the amino-acid balance. Dr Pal has rightly stressed the importance of developing wheat varieties with a high lysine content, since this is one of the limiting amino-acids in the average Indian diet. Thanks to the discovery of Professor Mertz and co-workers in the United States that the quality of proteins can be subjected to genetic improvement, studies of the kind proposed by Dr Pal are now feasible. The importance of such research can hardly be overemphasized since it is now known that protein malnutrition, particularly in the young, leads to a permanent impairment of the mental and physical faculties of the individual.

About 66 per cent of the wheat acreage in India is rain-fed. Hence the need for the development of varieties and agronomic techniques which would help to improve the productivity of wheat under *barani* (rain-fed) conditions is urgent. The book summarizes the excellent work done by Dr R. D. Asana and other physiologists in standardizing indices for the selection of drought-resistant varieties. The methods of water conservation and other appropriate agronomic techniques are also described. If the knowledge already available is applied in the form of a package programme for the rain-fed areas, there would be an immediate spurt in the yield of wheat in such regions.

Diseases, particularly rusts, take a heavy toll of the wheat crop. If their importance is not felt today, it is only because of the development and spread of varieties resistent to the major physiological races of the black, brown and yellow rusts prevalent in our country. The late Dr K. C. Mehta showed that rust infection in the Indo-Gangetic plains takes place through spores which come from the hills. Because of the high summer temperature in the plains, the spores formed in the plains do not survive. Hence, fresh infection takes place from the hills where the uredospores survive during summer. Most of the Pusa wheats are highly resistant to loose smut. The importance of resistance breeding will be more apparent in the coming years, since the improved conditions for the growth of the crop now being created would also promote the spread of diseases and pests. The book contains information on all the major and minor diseases of wheat and also on the few pests that are harmful. Diseases like *Alternaria* and *Helminthosporium* are also gaining in importance and need detailed study.

Very useful information is contained in the book on soils, agronomy and machinery as related to the cultivation of wheat. It is now well known that a high-yielding

variety possesses only a potential for yield and that the realization of this potential would depend on the agronomic and management factors practised. Also, an important cause for the low yield in India of all crops, including wheat, is the poor plant population. Unless improved seed-drills are used, and some degree of land levelling done, it would be difficult to ensure uniform germination and a good plant population. Hence, agricultural production needs attention to a whole set of interacting factors. In his 'Foreword' to the book Shri C. Subramaniam has drawn attention to this need in the following words : " If we are to grow more food on the existing land, the per-hectare productivity of our crops has to be greatly increased. This can be done only through the collaborative efforts of a team of scientists belonging to different disciplines, all striving to produce a plant with an integrated personality—by which I mean the ability of the variety to live in harmony with its environment and to use most effectively sunlight, water and nutrients. It would be futile to expect that any one input alone would help us to make a substantial gain in yield. For example, when a variety which is susceptible to rusts is grown with fertilizers, the increased incidence of diseases more than offsets the improvement in yield potential caused by the fertilizers. It is, therefore, necessary that the problem is viewed as a whole and all the forces made to interact in a manner that would help us to achieve the desired end-result. "

Recently, exciting developments have taken place in breaking the yield barrier in wheat through the introduction of the 'Norin' dwarfing genes and the evolution of a 'new agronomy'. To keep the book up to date, a brief addendum has been inserted, which describes the new era of wheat-improvement work in the country initiated in 1963, when the Indian Agricultural Research Institute introduced into our country a wide range of dwarf wheat material from Mexico with the help of the Rockefeller Foundation. The first systematic introduction took place only during the *rabi* of 1963–64, and during the *rabi* of 1966–67 nearly 400,000 hectares were under the dwarf wheats. This is sufficient evidence of the pace of progress in modern breeding, seed multiplication and developmental work. This book is hence a most valuable and timely publication.

M. S. SWAMINATHAN

The Rose in India. By B. P. PAL. Indian Council of Agricultural Research, New Delhi; pp. xviii+265; 25·5 cm×16·5 cm; 1966; Black-and-white illustrations 47, colour plates 46, line drawings 6; Price Rs 36·50; Postage Rs 2·50.

This wonderful book has been dedicated to Jawaharlal Nehru, who loved roses. There is a foreword by the Vice-President, and in the preface Dr Pal explains how he came to write the book.

Hitherto we have had to turn to literature from foreign sources and it has been all but impossible to follow the advice on growing roses because of the widely different soil and climatic conditions. This book has been written by one who has had forty years of practical, and successful, experience of rose cultivation.