

Citrus decline

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INTRODUCTION

Citrus decline, also often termed as citrus dieback, is not a specific disease, but a malady involving complexities of the indistinguishable disorders in the plant. It has also been referred to as flenching, decay, chlorosis, neglectosis, amachamiento in Mexico (Orozco-Santos, 103), citrus blight in Florida and declinio of citrus in Brazil (Wutscher *et al.*, 144). Citrus plantations decline in yield and growth following a period of satisfactory performance lasting for several years (Chadha, 31). This write up furnishes an account of the various factors associated with the decline syndrome and aims at highlighting the strategic measures to bring down the losses.

CITRUS DECLINE SYNDROME AND THE DIAGNOSIS

Symptoms of the citrus decline often vary with the cause of the malady. Accurate diagnosis is, therefore, an important pre-requisite. Generally, the trees perform well during the initial 5 to 6 years, but gradually thereafter, they show the retarded growth often rendering them uneconomical. Affected trees do not usually die out, but remain retarded and unproductive for a couple of the years. In extreme cases, they may wilt and die relatively rapidly. From a distance declining plants look sickly having one or more of the following symptoms.

- Earlier symptoms are restricted to a few limbs, but eventually the whole tree may be involved.
- The affected trees show sparse mottling of the leaves, stunted growth and a sickly appearance.

- Midrib and lateral veins of the mature foliage turn chlorotic with interveinal area along with the veins showing a diffuse yellowing.
- Chlorotic leaves are shed with the onset of the summer or autumn and the dieback of the twigs commences.
- Dead shoots look prominent and the dieback may progress right down to the main trunk.
- Entire periphery of the tree bears short twigs carrying the small leaves on their lower portion.
- Subsequent secondary growth from the axil buds consists of the weak, small, upright shoots, which may be bushy with green veins/ blotches on the foliage.
- The weak shoot shows various types of the discolouration on the leaves frequently resembling to those caused by the zinc and/or iron deficiency.
- Occasionally small, circular, green spots appear on the chlorotic tissue of the leaves.
- Excessive flowering is noticed, which produces sun blotched fruits that seldom mature.
- The sparsity of the foliage associated with the dieback affected shoots are invariably attacked by one or more fungi, which cause wood rot and ultimately lead to the destruction of the tree.

- Feeder root system becomes dark due to defoliation and may show rotting signs.
- There may be a few or almost all the trees under the decline in an orchard without depicting any definite trend.

The decline is not a specific disease, but merely a term, that denotes the wasting away the growth or fruit production and that may involve the ultimate death of the tree (Knorr *et al.*, 75 and Wutscher, 143). It is rather unfortunate, that the various ailments of the citrus in India have been brought together under one 'catch-all' term decline, thus, resulting in beclouding the problem rather than illuminating it.

A scanning of the voluminous literature available on the subject reflects, that a variety of the factors singly or jointly is responsible for the decline syndrome, namely, lack of the congeniality in a stionic combination, adaptability to the soil and climate, improper drainage, aeration, malnutrition, biotic stresses due to viral, fungal, bacterial, nematodal and insect-pest attacks etc. besides the faulty management of the orchards. The weakened plants are often more vulnerable (Reddy and Murti, 116).

According to the concept of the limiting factors, one of these factors will dominate over the others. For reaching a solution of the problem a systems analytical approach of tackling the contributing factor one by one is always desirable.

FACTORS RESPONSIBLE FOR THE DECLINE

Edaphic factors

Majority of the researchers have attributed the decline of the citrus trees to the inhospitable soil conditions (Haury, 60; Kanwar *et al.*, 69; Nilangekar and Patil, 102 and Randhawa *et al.*, 111 and 112).

The presence of the calcium carbonate layers or clay deteriorates the permeability of water and aeration of the soil as well as root growth and drainage. The calcium carbonate pan may occur at

the depth of 30 to 180 cm in certain soils of the Punjab and Haryana. Kanwar *et al.* (69) related the occurrence of the lime concretion layer upto 75 cm of the soil surface or within the feeding zone of the plants to the soil incongeniality in Punjab. According to them, calcium carbonate content in the citrus soil was responsible for the decreased availability of the iron and zinc producing their deficiency symptoms in plants. Surveys of the citrus soils in Punjab have indicated an association of the declining citrus orchards with >20% calcium carbonate concretions within the root zone. Similar observations have also been reported from Bombay (Bhatt, 27), Andhra Pradesh (Naidu and Krishan Rao, 92) and Punjab (Randhawa *et al.*, 112).

Singh (128) and Bhambota (25) attributed the decline syndrome to the salinity in soils of the Sangrur district in Punjab. However, Joolka *et al.* (66) found no harmful effect of the saline water irrigation on the growth of the Blood Red/Jatti Khatti combination upto 2.5 d Sm⁻¹. However, Bhambota and Kanwar (26) recorded the harmful effects with 500 ppm sodium chloride water on the growth of the Blood Red/Rough lemon. A water salinity of 8 d Sm⁻¹ proved lethal to a number of the rootstocks.

Citrus is highly sensitive to the excessive salt concentration. Citrus trees are injured, if the total salt concentration exceeds 1,000 ppm. Soil salinity has been reported to be the main cause of the chlorosis of citrus trees in Punjab (Dhingra and Kanwar, 48).

Citrus grows best in a pH range of 4.5 to 8.5 and the pH of > 8.7 is considered excessive (Randhawa, 110). High sodium content may prove toxic to the citrus. The critical limit of the exchangeable sodium percentage (ESP) for the light textured soils of the Punjab and Haryana may be taken as 20 (Randhawa, 110).

In the recent past, there has been a rise in the water table probably due to intensive canal system of the irrigation in Punjab, Haryana and Rajasthan and the network of the rails and roads obstructing natural drainage. Inadequate drainage is one of the

important factors attributed for the citrus decline in different parts of the country (Bhatt, 27 and Kanwar and Randhawa, 70). Defective drainage has been reported to disturb the nutritional balance in citrus trees besides limiting the oxygen supply to the roots and production of the harmful anaerobic products.

The soils having >5% lime in any of the profiles upto 180 cm depth was conducive for the decline. Many soils are deteriorating because of the low organic matter or high ESP (Pinckard, 106).

Rootstocks

The rootstocks provide tolerance/resistance to the biotic and abiotic stresses besides influencing the yield, quality as well as the keeping quality of citrus fruits. Each scion variety reacts and responds differently to the same type of the rootstock with respect to the soils, climates and viruses. Therefore, no universal rootstock can be recommended, which would be suitable for all the varieties under varied situations.

The rootstock situation in India has been reviewed adequately (Agarwal, 5; Aiyappa and Srivastava, 15; Chadha, 31; Dhatt and Singh, 44 and Randhawa and Srivastava, 109). The overall rootstock scenario in the country reveals a replacement of the rough lemon with Rangpur lime in some states. Although, it is susceptible to the root rot under laboratory conditions (Iyer *et al.*, 63), it provides longevity to the scions. The susceptibility of the Rangpur lime to the exocortis virus will, however, necessitate the use of the exocortis free scion material.

Resistant or tolerant rootstocks against several viruses and mycoplasma diseases have been identified (Ahlawat and Sardar, 8; Ahlawat *et al.*, 12; Cheema *et al.*, 37; Fraser and Singh, 50; Naidu and Ramakrishnan, 91 and Nariani *et al.*, 94), but their horticultural suitability needs to be evaluated.

In several instances, the citrus trees decline because of the graft incompatibility e.g. Mosambi on *Jambhiri* in Bombay state (Nagpal, 90), Sathgudi on *Jambhiri* in Andhra Pradesh (Chadha and Singh,

32), Nagpur mandarin on *Kandhia local* or *Karuan* in Orissa and Blood Red and Mosambi on rough lemon in Punjab. The predominant rootstocks in different citrus growing states are *Jatti Khatti* (Punjab, Haryana, Himachal Pradesh and Rajasthan), *Kharna Khatta* (Uttar Pradesh), Rangpur lime for Mosambi (Maharashtra) although, rough lemon strains of the *bulliki-chilli* and *Kharna Khatta* also do well (Chadha, 31). In Mysore region, rough lemon and the Rangpur lime have emerged as potential rootstocks for the Coorg mandarins. Horticultural performance and reaction to the biotic and abiotic stresses of the common rootstocks are given in Table 1 on page 89.

Due to prevalence of the viruses, salinity and nematode problems in different regions, it is highly desirable, that the citrus rootstocks are thoroughly evaluated. Biochemical and physiological basis of the compatibility/incompatibility should be worked out to minimize the testing time and to safeguard against the failures.

Nutritional factors

Improper and inadequate nutrition (Table 2) forms one of the main causes of decline in India (Anonymous, 1; Bhatt, 27; Chadha, *et al.*, 33; Iyer and Iyengar, 64; Ramakrishnan, 108 and Singh and Bakhshi, 131).

Most of the Indian soils are low in organic matter and available nitrogen (Bojappa and Bhargava, 29). Therefore, citrus responds well to the applied nitrogen. Iyer and Iyengar (64) found, that when the soil pH is below 6 (Coorg, Karnataka), nitrogen availability is less and deficiency appears, resulting in reduced girth and height and more general chlorosis (Aiyappa *et al.*, 16) According to Hossain *et al.* (61) application of the nitrogen to the declined/declining orange trees resulted in a marked growth improvement.

According to Saini *et al.* (124), the declining trees had low level of the nutrients (N, P, K, Ca, S, Zn, Cu, Fe, and Mn) and more incidence of the pre-harvest fruit drop than the healthy trees. The fruit drop is known to aggravate the tree decline

Table 1. Characteristics of some selected rootstocks

Rootstock	Horticultural performance				Reaction to the stresses					
	Yield	Quality	Plant vigour	Root system	Biotic stress			Abiotic stress		
					Root rot	Exocortis	Tristeza	Citrus nematode	Salt	Drought
Cleopatra	M	M	M	M	T	R	R	S	MT	D
Carrizo citrange	M	G	M	SH	MT	S	MT	T	HS	HS
Sour orange	G	G	M	D	R	T	HS	T	T	MT
Kharna Khatta	G	M	M	D	S	T	-	MT	T	S
Marmalade orange	G	M	G	D	MT	S	R	MT	R	R
Nasnaran	M	G	M	M	S	R	T	MT	HT	S
Rangpur lime	G	M	G	D	MT	S	R	S	R	R
Rough lemon	G	L	G	D	S	R	R	S	T	T
Sweet lime	M	M	G	M	S	S	S	MT	S	S
Sweet orange	G	G	M	M	HS	R	MT	HS	S	S
Trifoliate orange	L	M	L	D	R	H	R	R	HS	HS
Troyer citrage	M	G	M	SH	MT	S	MT	T	HS	HS

G = Good, M = Moderate, L = Low, R = Resistant, T = Tolerant, MT = Moderately tolerant, S = Susceptible, D=Deep M = Medium, SH = Shallow, - = No information
Chadha and Singh, 32.

Table 2. Range of the effects produced by the nutrient stress in citrus

Nutrient stress	Effect produced	Reference
Nitrogen (ammonical)	Depressed absorption of the potassium by the young orange seedlings.	Bojappa and Bhargava (29).
Phosphorus content in the soil	Negative relationship with the potassium in the foliage.	Murthy <i>et al.</i> (89).
Superphosphate application	Depressed growth of the citrus roots.	Bojappa and Bhargava (29).
Excessive application of the potassium	Magnesium deficiency symptoms, increased level of the calcium and magnesium in the soil, decreased uptake of the potassium.	Randhawa and Srivastava (109) and Singh (129).
Deficiency of the potassium	Increased susceptibility to the cold and drought.	Bojappa and Bhargava (29).
Subnormal calcium	Increased susceptibility to the fungal infection.	Chapman (34) and Randhawa and Srivastava (109).
Excessive copper accumulation	Deficiency of the iron.	Bojappa and Bhargava (29).
Zinc and iron deficiency	Masking of the manganese deficiency symptoms.	Bojappa and Bhargava (29).
Excessive copper sprays	Induction of the zinc deficiency.	Bojappa and Bhargava (29).
High boron content	Increased granulation in the sweet orange.	Munshi <i>et al.</i> (88).

because, the dropped fruits have already drawn the metabolites from the tree and exhausted it (Mullar, 86).

Although, most of the Indian soils are well supplied with potassium, regular omission of the potassium in fertilizer schedules leads to a low content of the potassium in leaf and subsequent decline in fruit yield and quality. Sekhon *et al.* (126) reported the low calcium content in 29 per cent of the declining sweet orange trees in Ferozepur and Bathinda districts of Punjab mainly due to excessive potassium status.

Nauriyal *et al.* (95) reported, that the citrus die back might be due to calcium deficiency. Kanwar *et al.* (68) reported 3.2% calcium in healthy and 2.6% in chlorotic leaves. Similar results were also reported by Randhawa *et al.* (111) and Nair *et al.* (93). Several workers have demonstrated the efficacy of zinc sulphate in combating the die back and mottling syndrome of the citrus (Singh and Agarwal, 132).

In Florida, the term die back is associated with the deficiency of copper. In India, due to frequent sprays of the copper fungicides like Bordeaux mixture and blitox for the disease control, copper deficiency is seldom noticed. In Columbia, the declining trees showed high zinc content (77 ppm) and slow water uptake (0.97 ml/min), reported by Huertas *et al.* (62).

Deficiency of boron is a problem in the light sandy and acidic soils. Its deficiency seldom occurs in the alkaline soils, where boron excess affects the growth and productivity. In severe boron injury, the leaves fall prematurely, eventually, resulting in quick die back syndrome (Randhawa and Srivastava, 109).

The inherent low organic matter in soil under the tropical and subtropical climates and due to high pH, micro-nutrient availability becomes a major limiting factor. Antagonistic relationship between the micro-nutrients like iron-manganese, iron-zinc, iron-copper, copper-molybdenum etc. makes detection and correction of the disorders

difficult. The deficiency symptoms of one element may superimpose others or those due to fungal and/or viral diseases. Wide spread micro-nutrient deficiencies occur in different states (Bojappa and Bhargava, 29), which have been attributed to a low available nutrient status, free calcium carbonate and high phosphorus status in soils. The deficiencies of the micro-nutrients occur in various combinations (Table 3). The disorder should be identified by visual diagnosis supported by soil and foliar analysis before recommending the nutrient sprays. Majority of the orchardists do not follow the micro-nutrient application and their orchards show the hunger signs.

Orchard management

There are several instances, where citrus decline may be traced back to the negligence and mismanagement of the orchards. Factors like inadequate tree spacing, improper manuring, nutritional disorders, defective irrigation, intensive intercropping and improper management of insect-pests and diseases are often responsible for this malady.

The commercial citrus species, viz. mandarin, sweet orange and lime are planted at 6 x 6 m apart to provide an adequate scope for intercropping during the initial 4 to 5 years. However, excessive intercropping has led to the decline in citrus orchards in Punjab, Haryana and Rajasthan. From an exhaustive review of the intercropping in orchards (Chundawat, 41) several profitable, shallow rooted, short duration and compatible intercrops for the citrus have been indicated, which do not shade the trees or harbour insect-pest and diseases of the citrus. There are report of the severe infestation of the root-knot nematodes (*Meloidogyne sp.*) in citrus due to tobacco and okra growing as intercrops (Perraju, 105 and Tirmula Rao, 139). The solanaceous vegetables like tomato, brinjal and chillies, which are known to favour the nematode infestation should not be encouraged as intercrops (Rao *et al.*, 114) and legumes should be preferred but with an adequate care to manage the aphids.

Table 3. Various combinations of the multiple deficiencies in some citrus species

Citrus species	Deficiency of the micro element(s)	Reference(s)
Sweet orange	Zn, Cu, Fe and Mn	Anonymous (2)
	Zn	Kanwar and Dhingra (67)
		Sekhon <i>et al.</i> (126)
	Zn and Cu	Kumar and Sharma (76)
	Zn and Mn	Nair <i>et al.</i> (93)
		Sharma <i>et al.</i> (127)
	Zn, Cu and Mn	Malewar <i>et al.</i> (80)
Mandarin	Zn, Cu and Mn	Marudarajan (83)
	Mg, Zn and Mn	Ramakrishnan (108)
	Zn and Mn	Dikshit (49)
		Wallace <i>et al.</i> (142)
	Zn and Fe	Chaudhary (35)
		Jhadav <i>et al.</i> (65)
Kagzi lime	Zn and Cu	Singh and Agarwal (132)
	Zn	Malewar <i>et al.</i> (80)
		Nijjar and Brar (101)
	Zn and Mn	Reddy and Sharma (117)
	Zn	Reddy and Sharma (118)
Raja (107).		

There are chances of the chlorosis in citrus trees due to intercrops requiring frequent irrigation (Azad and Bhambota, 17), such as potato followed by berseem, lucerne, tomato, brinjal, *senji*, wheat, bittergourd and gram in a decreasing order.

Non-reclamation of the multiple micro-nutrient deficiencies has been considered a major factor responsible for the citrus decline (Chadha *et al.*, 33). It is desirable that the citrus orchardists get their soil tested once in five years, so that the proper manuring schedule may be implemented. A careful watch on the micro-nutrient deficiencies has to be kept (Reddy and Murti, 116). Many citrus soils are showing a low organic matter content or high exchangeable sodium. These need to be corrected by applying the bulky organic matter like farmyard

manure or compost and gypsum (Randhawa, 110).

Citrus trees are quite sensitive to the water stress during their active growth, flowering and fruit set. According to Dhillon and Singh (45) water stress is detrimental to the fruit retention at the initial stages. Over irrigation and water logging often cause leaching of the essential nutrients (Ghosh, 58) and predispose the trees to the root rot disease (Fraser and Singh, 50). Better drainage in the orchards must be ensured as over irrigation leads to the decline (Rao, 113) and wilt in the nursery (Bedi, 23). Light and frequent irrigations are recommended by Bakhshi and Singh (19) to get an optimum yield, because excessive use of the water leads to the root decay and decline (Chadha, 31).

Incidence of the virus and virus-like diseases

Citrus is prone to more than 77 virus and virus-like diseases, which have the potential to debilitate or destroy the citriculture as a viable venture from a region or a country. Either new diseases are appearing or those hitherto unimportant are becoming the cause of concern (Table 4). These diseases reduce the fruit size, its quality and yield, induce nutritional problems, increase susceptibility to the frost injury, induce bud-union problems rendering certain, otherwise desirable, rootstocks unfit and shorten their life expectancy in the form of the decline (Bar-Joseph and Oren, 21; Chenulu and Ahlawat, 39; Kapur and Sohi, 71 and Kapur and Cheema, 73).

In India, virus and virus-like pathogens, which have been found associated with die back, are

crinkly leaf, infectious variegation, exocortis, leathery leaf, mosaic, tristeza, rubbery wood and greening (Ahlawat and Raychaudhury, 11). Other diseases, which have been recorded/established, are psorosis-S, impietratura (Ahlawat *et al.*, 13), woody gall (Mali *et al.*, 81), leaf curl (Mali *et al.*, 82) and yellow vein disease (Rustem *et al.*, 123). A positive correlation between the age of the trees and the incidence of the greening, tristeza and mosaic has been observed in Coorg mandarin at Gonicoppal in Karnataka, Kinnow and sweet orange in Punjab and limes in Andhra Pradesh.

Tristeza

Tristeza (sadness in Spanish and Portuguese) caused by citrus tristeza virus (CTV) originated in the Orient and got distributed worldwide by movement of the citrus budwood (Bar-Joseph *et*

Table 4. Newly emerging virus or virus-like diseases in different parts of the world

Disease	Country	Reference
Citrus variegated chlorosis (CVC)	Brazil	Rossetti <i>et al.</i> (122) and Lee <i>et al.</i> (77).
Witch's broom of lime	Oman and UAE	Garnier <i>et al.</i> (54).
Leathery leaf	India	Ahlawat <i>et al.</i> (12)
Measles	Brazil and Florida	Lee <i>et al.</i> (78).
Rubbery wood	India	Ahlawat (6).
Severe citrus mosaic	India	Ahlawat <i>et al.</i> (14).
Yellow vein clearing	Pakistan	Catara <i>et al.</i> (30).
	India	Rustem <i>et al.</i> (123).
Yellow spotting on the foliage of the naval orange	Spain	Navas-Castillo and Moreno (97 and 98).
A new Rhabdo virus in the grapefruit and pummelo	India	Ahlawat <i>et al.</i> (10).
A new *stem-pitting strain of the tristeza	Indonesia, Malaysia and Thailand	Tsai-Mei-Chen <i>et al.</i> (140).
Citrus ring spot virus (CRSV)	India	Thind <i>et al.</i> (138).
Lemon crinkling leaf virus	India	Ahlawat and Sardar (8 and 9).

* Possesses virulence to attack normally the tolerant mandarins in Indonesia, Malaysia and Thailand.

al., 22). Several epidemics have occurred in Argentina and Brazil in thirties, when over 30 million citrus trees were killed (Costa, 42) and in Spain during sixties and in Venezuela during eighties, when 10 and 6.6 million trees were killed respectively (Rocha-Pena *et al.*, 119). Less severe epidemics have also occurred in Israel, California and Florida. Natural occurrence of the CTV is also known in India (Ahlawat and Raychaudhury, 11). The highest incidence of the CTV has been reported from Tamil Nadu (71%) followed by

Karnataka (65.3%) It is, thus, considered to be one of the main causes of the citrus decline in India (Ahlawat, 7).

CTV is transmissible by grafting but not by the seed. Its mechanical transmission is difficult by inoculation of the partially purified preparations into the stems of the sensitive indicator plants (Garnsey and Muller, 57). Its host range is largely restricted to the Rutaceae family (Citrus species and relatives) with an exception, that it may infect *Passiflora gracilis* (Muller *et al.*, 87).

Table 5. Distribution of the virus and virus-like diseases in India

Virus/virus like-disease	Distribution
I. Common and devastating	
Tristeza including seedling yellow strain	Throughout India
Greening	Throughout India
II. Serious in some of the regions	
Mosaic	Serious in AP reported from A and MR
Exocortis	A, K, MR and Pb
Psorosis	A, K and MR
III. Diseases of the minor importance	
Vein enation and woody gall	A, K, MR and TN
Infectious variegation	AP and NER
Multiple sprouting	NER
Xyloporosis	MR
Leaf curl	MR and NER (Sikkim)
Yellow corky vein	AP, A and K
Leathery leaf	WB (Darjeeling)
Oily specks	AP, K and MR
Impictatura	K and Pb
Blastomania	MR and K
Rubbery wood	Sikkim
Wound tumour	MR and NER

A = Assam; AP = Andhra Pradesh; K = Karnataka; MR = Maharashtra; NER = Noth-Eastern Region; Pb = Punjab; TN = Tamil Nadu; WB = West Bengal
Ahlawat and Raychaudhury (11).

A comparison of the international isolates of the CTV and their description has been worked out (Garnsey *et al.*, 55 and 56). Different strains of the CTV cause strikingly different symptoms, such as quick decline, slow decline, stunting or no symptoms (Adams, 4). The mild strain is prevalent in citrus species and varieties in northern Indian plains, which might be rendering protection against severe and devastating strains (Ahlawat and Raychaudhury, 11). Severe strains have been detected in the samples from Assam, Meghalaya, West Bengal in the plantations of the grapefruit, lime, lemon and pummelo (Table 5). The severe strains and the seedling yellows were restricted to the areas, where a vector was active. The strains may be identified on the basis of ELISA tests (Azeri, 18).

Stem pitting forms of the CTV cause the limbs and twigs of citrus trees to develop pits, that interrupt the nutrient carrying ability of the limbs, which subsequently leads to the decline (Klein, 74). Control strategies for the management of the CTV should include immediate efforts, such as :

- Preventing further introduction and spread of any severe strain into the region;
- Continued education of the growers and the nurserymen;
- Eradication, where number of the infected trees are less;
- Avoiding long range mobility of the brown citrus aphid infested plant material;
- Field evaluation of the alternate rootstocks in the areas, where sour orange is threatened by the CTV;
- Mild strain cross protection against the severe strains;
- Regular indexing to know the status of the existing plantation with respect to CTV and
- Development of the immune scion varieties through genetic engineering and breeding.

Greening

It is caused by a bacterium (*Liberobacter asiaticum*) and devalitizes the citrus industry in

North-West India. The African strains are heat sensitive, while the Asian ones are heat tolerant. Therefore, they are difficult to manage. Three categories of the strains i.e. severe ($G_1 - G_5$), moderate ($G_6 - G_8$) and mild ($G_9 - G_{12}$) have been identified from Punjab (Singh *et al.*, 133). Cross-protection ability of the mild strains ($G_9 - G_{12}$) have been evaluated through bud inoculation of a Pineapple sweet orange against the challenge inoculation of a severe strain G_2 . Though, the cross-protected seedlings were taller with more girth, leaf area and internodal distance, yet the biochemical differences were not perceptible. According to Wynd (145), the virus infection alters the permeability of the cytoplasm, so that the soluble substances diffuse more slowly in the infected cells than in the uninfected ones. The virus infection decreases the rate of the starch synthesis and translocation (Thind, 137). Therefore, starch is retained in the infected areas, because the enzymes responsible for transforming the starch into sugars and vice-versa are inactivated.

The role of the greening disease in citrus decline is all the more alarming, because of its efficient citrus psylla vector for the development, for which, the arid climate of Punjab, Haryana and adjoining Rajasthan is quite congenial. The inoculum reservoir is easily maintained in the range of citrus species, which are often grown in the backyards of the houses.

Much success has not been achieved in controlling the citrus diseases through chemicals. A good deal of the work, however, has been done for combating the greening pathogen in the infected citrus trees. Tetracycline, penicillin and bavistin have been reported to suppress the symptoms of the greening, when applied either through bud-dip treatment (@500 µg/ml), foliar sprays (@500 µg/ml) or trunk injection (Kapur and Sohi, 71). An integrated application of the tetracycline and fertilizer management also led to an increase in the yield and tree volume and a marked reduction in the chlorosis in Kinnow. Orozco-Santos (103), however, did not find tetracycline injection effective.

Psorosis

Psorosis is a wide spread and damaging disease of citrus in many parts of the world including South America and the Mediterranean regions (Roistacher, 121). Chlorotic flecking and ring spot may be produced in the leaves of infected trees. Two types of the psorosis, A and B, have been distinguished, which are different to each other in their symptoms, but related to each other according to the evidence from the cross-infectivity tests (Garcia *et al.*, 53). According to Ahlawat (7), psorosis is more serious in Karnataka (40%) and the chlorotic ring spot in Punjab and Delhi (50 to 80%). The incidence of these diseases is higher in the declining trees as compared to the healthy ones. Since, psorosis is spread by the infected bud wood, control must be efficiently carried out in nursery. The use of the bud wood known to be free from the causal virus, the trees may be grown, which would continue to remain psorosis free (Knorr *et al.*, 75).

Production of virus free planting material

Propagation materials are the chief source of dissemination of many viruses and virus-like diseases associated with the decline syndrome. For their management, foundation block (FB) of the disease free bud wood sources and a clean nursery programme are essential. Though, the virus free plant may also get infected in the field in due course of the time, yet the stage at which the first infection occurs has a definite bearing on the loss. Once the plant is established, it may sustain the losses under a good orchard management system. On the other

hand, a virus infected tree may not reach the production stage at all. It has been observed during the surveys, that the orchards planted with healthy trees were highly productive with prolonged orchard expectancy as compared with those orchards where care was not exercised for planting the healthy material. Use of the certified disease free plant material is important for preventing the virus and virus-like disease epidemics (Deol, 43). Production of the virus free quality plants would involve a number of the steps, like selection of the mother trees, detection of the virus/virus-like diseases, nuclear foundation stock, cleaning viruses through shoot-tip grafting and the bud wood certification (Singh, 130).

Role of indexing

It has successfully been employed for the detection of the viruses/virus-like agents in citrus plants. About 17 viruses/virus-like agents associated with citrus decline may be identified through indexing on the indicator plants (Anonymous, 3). The citrus greening bacterium and exocortis viroid may also be detected through biochemical indexing (Fudl-Allah *et al.*, 52 and Schwarz, 125), which is more reliable, time saving and an economical approach (Anonymous, 3). Citrus greening pathogen may be detected within 48 hours (Schwarz, 125) whereas, all the above ground plant parts may be used for its detection (Cheema and Kapur, 36 and Cheema *et al.*, 37). The indexing on the indicator plants may be short term (10 to 12 months) or long term indexing (3 to 4 years) against different viruses (Table 6). The indexing (Table 7)

Table 6. Indexing on the indicator plants

Indexing	Period	Virus/virus-like diseases
Short term	10 to 12 months	Cirtus tristeza virus Citrus exocortis viroid Citrus greening bacterium
Long term	3 to 4 years	Xyloporosis Citrus podagra virus Citrus gummy bark virus

Anonymous (3), Cheema and Kapur (36) and Kapur *et al.* (72).

Table 7. Results of the indexing against the citrus tristeza virus in the citrus growing areas in India.

State	Cultivars	Plants showing reaction (%)		
		Mild	Severe	Seedling yellow
Andhra Pradesh	Mandarin, Satgudi orange and lemon	32.0	4.0	-
Arunachal Pradesh	Khasi orange and Local sweet orange	40.0	-	-
Assam	Khasi orange, Washington Naval, grapefruit, Assam lemon, Sour orange and pummelo	50.7	9.6	-
Delhi	Mandarin, Mosambi, Kagzi lime and grape fruit	13.3	-	-
Haryana	Malta and Kagzi lime	3.3	-	-
Himachal Pradesh	Kinnow, Cleopatra mandarin and Kagzi lime	8.0	-	-
Meghalaya	Khasi orange, Washington Naval, grapefruit and Local lemon	26.7	2.2	2.2
Rajasthan	Kinnow mandarin, lemon, Kagzi lime and Mosambi	35.7	5.7	-
Sikkim	Sikkim orange	40.0	5.0	-
Uttar Pradesh	Mandarin, Mosambi, lemon, Kagzi lime; Satsuma mandarin and sweet lime	13.9	-	-
West Bengal	Darjeeling orange, Kagzi lime, lemon and pummelo	52.2	2.2	-

Ahlawat and Raychaudhury (11).

of the citrus cultivars in citrus growing areas of India against CTV indicated, that the mild strain is most prevalent throughout the country, whereas, severe strains are found in the southern and north-eastern regions of the country (Ahlawat and Raychaudhury, 11).

Bud wood certification programme

For the supply of virus free plants and bud sticks to the growers, bud wood certification programmes have been designed in India and elsewhere (Dhillon *et al.*, 46 and 47; Nijjar and Kapur, 100). Apparently healthy, vigorously growing, true-to-type citrus plants are selected as the candidate trees. Bud wood from such trees is bud-inoculated to various sets of the indicator plants for indexing against the viruses and virus-like agents. Simultaneously, primary increase trees (PIT) are propagated by budding on the rough lemon or

other suitable rootstocks raised under the insect proof cages. If symptoms do not appear on any of the indicator sets, PITs are reindexed for raising the foundation block (FB), which are used for the preparation of the mother plants (MP). These blocks are regularly subjected to the reindexing to confirm, that they are free from the viral infection. All the FB and MP trees, those have passed the indexing and the visual inspection tests, are given a registration number, and thus, a regular supply of the certified virus free plants and bud wood for the growers is regulated.

There are number of diseases of the citrus, which if introduced may seriously hamper its performance. These include exotic strains of the tristeza, greening, canker, blight, witches broom, citrus variegated chlorosis and fruta bolita (Roistacher, 120). This demands, that the certificate programmes should be mandated not only to

prevent their introduction into the citrus growing areas but also to prevent their dissemination into the main stream of the bud wood supply. The steady growth and the expansion of the air traffic increase the chance, that these destructive citrus diseases might be introduced.

Some graft transmissible diseases may get introduced in the new areas along with symptomless hosts (Table 8) and may seriously debilitate the citrus industry. Strict policies mandating the certification would help in preventing the movement of the diseases (Roistacher, 120).

concern for citrus particularly in low lying and damp areas. *P. citrophthora* and *P. nicotianae* var. *parasitica* cause a drastic loss in production. Use of the resistant rootstocks is considered the most effective way of controlling this problem. Methods of assessing the citrus seedlings to damage by the *Phytophthora* and salinity, both in the intact plants and callus culture, have been developed (Sulistyowati and Keane, 135 and 136).

Two species of the nematodes are known to be destructive for citrus. *Tylenchulus semipenetrans* was earlier demonstrated to be responsible for the "slow decline" of the citrus in California. The other

Table 8. Graft transmissible diseases of the citrus, which may be introduced as symptomless carriers

Disease	Causal organism	Symptomless host
Tristeza	Citrus tristeza virus	Mandarin and sweet oranges.
Greening	<i>Liberobacter asiaticum</i>	Lime.
Witches broom	MLO	Lime or possibly other species.
CVC	<i>Xylella fastidiosa</i>	Several citrus species.
Exocortis	Citrus exocortis viroid	Sweet orange, mandarins, limes and lemons.
Cachexia	Citrus cachexia viroid	Sweet orange, grapefruit, limes and lemons.
Tatter leaf	Citrus tatter leaf virus	Most citrus cultivars.
Concave gum	Virus	Lemon and grapefruit.
Psoriasis-A	Virus	Most citrus cultivars.

Roistacher (120).

Other biotic stresses

In addition to the incidence of the viral and virus-like diseases, the fungal pathogens like *Colletotrichum gloeosporioides*, *Curvularia tuberculata*, *Diplodia natalensis* and *Fusarium* species have also been found to be associated with the citrus decline syndrome. The injured tissue of the citrus is often more susceptible to these pathogens, which develop fast after the rains. *Colletotrichum gloeosporioides* occurs in the latent form in apparently healthy twigs (Raychaudhuri, 115). In recent years, foot rot due to the *Phytophthora* spp. has become a cause of the

nematode, *Radopholus similis* or the burrowing nematode was the cause of very destructive "spreading decline" in Florida. Both have been reported to be present in India (Mukherjee and Dasgupta, 85).

The major pests of the citrus, contributing towards the decline, are citrus psylla (*Diaphornia citri*), leaf miner (*Phyllocnistis citrella*), green scale (*Coccus viridis*), black fly (*Aleurocanthus woglumi*) and mealy bug (*Planococcus citri*).

Citrus psylla is a known vector for the greening disease. Leaf miner is known to aggravate the

problem of the canker scab complex (CSC) thus, promoting the decline process. Backfly is a serious pest in Nagpur region of the Maharashtra and some parts of the Karnataka.

For these biotic stresses, the control strategies have already been worked out by several workers in India and abroad. The recommended strategies should be followed to mitigate these problems.

Nucellar embryony

The bud wood obtained from the nucellar embryos is virus free and meet the requirements of the certification (Dhillon *et al.*, 46). The selection of the nucellar seedlings may be accomplished by the control pollination with a marker pollen and the subsequent selection of the true-to-type seedlings (Deol, 43). The use of *Poncirus trifoliata* imparts the dominant trifoliolate leaf character to all the hybrid seedlings, which should be rejected. The method is quite suitable for producing the plants free from the xyloporosis, psorosis and greening (Dhillon *et al.*, 47), but the problem of the exocortis is not eliminated as *P. trifoliata* is susceptible to it.

Tolerant rootstocks

Diseases like tristeza, exocortis and leathery leaf affect certain stionic combinations. The use of the resistant/tolerant rootstocks may help to minimize these problems (Table 9).

Role of the biotechnological tools

The progress made in this area during the last 20 years has been adequately reviewed by Meunier (84). Paiva *et al.* (104) obtained a virus free cv. Seleta Folha Murocha (SFM) through micro grafting. A microscopic segment from the SFM plant into a micro rootstock obtained from the seed of the Rangpur lime. Navas-Castillo *et al.* (99) established and maintained the callus culture from the citrus explants infected with several viruses like Psorosis-A (isolate 121), Psorosis-B (isolate PB-108), ring spot (isolate RS-SR), crista cortis (C-601) or concave gum (CG-207). Such techniques may usefully be employed for the indexing as seedling inoculation of the sweet orange cv. Pineapple or grapefruit cv. Duncan with callus pieces in green house produced adequate symptoms. *In vitro* shoot tip grafting using different scion/rootstock combinations indicated, that the *Citrus sinensis* cv. Mosambi / *C. depressa* combination resulted in 19% successful micro grafts, whereas, *C. nobilis* × *C. deliciosa* hybrid Kinnow/*C. jambhiri* combinations resulted in 15 per cent successful micro grafts (Grewal *et al.*, 59). Micro grafting technique may be indexed against CTV and the greening in an insect proof cage.

In recent years, molecular diagnostics have emerged as a powerful tool. Nucleic acid probes have been shown to be more promising than the

Table 9. Rootstocks tolerant against the viruses and/or virus-like agents.

Virus/virus-like diseases	Tolerant rootstocks	References
Tristeza	Rough lemon and Cleopatra	Balaraman and Ramakrishnan (20) and Cheema <i>et al.</i> (38)
Exocortis	Rough lemon, Cleopatra, mandarins, sweet orange and sour orange	Cheema <i>et al.</i> (38), Chohan and Knorr (40) and Kapur <i>et al.</i> (72)
Xyloporosis (Cachexia)	Rough lemon, Cleopatra, mandarins, sweet orange and trifoliolate	Chohan and Knorr (40)
Greening	Rough lemon strains, Milan, Miri, Vollamar and South Africa-I and II	Cheema <i>et al.</i> (37)

use of ELISA (Vani, 141). There are several techniques developed for the amplification and the subsequent detection of the target viral nucleic acid, e.g. PCR and 3 ST, both of which are powerful in detecting as low as one molecule of the target viral genome in a sample consisting of a mixture of the heterogeneous viruses and a million molecules of the plant genome. The utility of these techniques depends on the availability of the actual sequence information.

The generation of the disease free stocks has acquired a significance in the cultivars, those have been vegetatively propagated for over generations. Viruses are difficult to eliminate by conventional methods. The techniques like nucellar seedlings, shoot tip culture, shoot apical meristem culture,

shoot tip grafting and regeneration of the plants from the cultural cells are available (Chenulu and Ahlawat, 39). The method of the shoot tip grafting has been extremely useful in citrus industry for generating the virus free planting material (Bitters *et al.*, 28 and Navarro, 96).

Integrated approach for decline management

For the management of the multi-factorial problems like citrus decline, an integrated strategy, involving multi-disciplines, like horticulture, ecology, plant pathology, entomology, soils, biotechnology, toxicology etc., is required (Fu *et al.*, 51). The management strategies for the low yields and short life span of the Taiwanese citrus due to greening disease, citrus tristeza and tatter leaf viruses (Su *et al.*, 134) include integrated

Table 10. Citrus management schedule in Taiwan

Month	Crop status			Management and target pest
January				Pruning, sanitation, control of the aphids, spring whitefly, scale insects and mites
February	S			Organic fertilizers
March	S	B		Irrigation and weeding
April		B	F	Leaf miner, aphids, root nematode and spiny whitefly
May			F	Thinning, fertilizers (P and K), black spot, long horn beetle and spider/rust mite
June	S		F	Aphids, leaf miners and psylla
July	S		F	Rust mites, thrips, stink bugs, spider mites and melanose
August			F	Weeding
September	S	H	F	Fruit fly
October	S	H		Collect fallen fruits
November		H		Harvest and pre-harvest spray before four days of the harvest
December		H		Sanitation, dipping for the storage (green mould rot and stem end rot)

S = Shooting or flushing, B = Blooming, F = Fruiting and H = Harvesting.

Su *et al.* (134)

control measures having a national programme of the pathogen free foundation stock and propagation of the healthy seedlings, pre-inoculation with CTV avirulent strains, field sanitation and insect vector control by insecticide sprays supplemented with the releasing parasitoid, *Tamarixia radiata* (Table 10).

Berg (24) recommended various steps, such as restriction of the citrus growing in hot and low lying areas of the South Africa, strict chemical control of the vectors in nursery, planting orchards with one cultivar of the same age and the same flushing periods. Luis *et al.* (79) showed significant social and ecological benefits from the adoption of a five component IPM system, including the key technical subsystem, information, decision making, extension and feedback subsystem. Use of the healthy plant material, elimination of the common host plants and application of the tetracycline in the Mediterranean regions are some other recommended practices.

FUTURE THRUST AREAS OF RESEARCH

For devising any such system for the decline management, a proper understanding of the different contributing problems would be a pre-requisite, so that each one is separately dealt with in terms of a system analytical approach.

For the better resource and information utilization, it is necessary to have a national coordination centre on the citrus decline research. This centre should initiate multi-location trials for developing the region specific recommendations. A comprehensive work on the survey and the surveillance of the problem needs to be done in different agro-ecological zones of the country.

Since, there is a regional peculiarity for the cultivars and also for the viruses, e.g. Punjab has more problem of the greening and exocortis, while mosaic in sweet orange and tristeza in acid lime are the problems in Andhra Pradesh. Vector population and transmission are also region specific. Therefore, a skilful regional planning is a need of the day.

In the present scenario, horticulturists, plant pathologist, entomologists, weed science experts and the scientists representing other allied disciplines including nurseries are working in isolation and that is why any foolproof, economically viable, socially acceptable and eco-friendly technology has so far not been developed against the citrus decline complex. A comprehensive scientific interaction should be planned to give ample scope to the scientists to deliberate and arrive at some solutions and the future thrust areas to herald novel interdisciplinary approach for an integrated management strategy against the citrus decline.

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