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Citrus Diseases

■ GUMMOSIS AND ROOT ROT

Species of *Phytophthora* cause the most serious soil-borne diseases of citrus throughout the world (205). Losses occur from damping off of seedlings in the seed beds, root and crown rot in nurseries, and from diseases variously known as gummosis, brown rot gummosis, brown rot, trunk rot, foot rot, collar rot, root rot, fibrous root rot, leaf fall and fruit rot. Serious losses can occur in orchards with trees on susceptible rootstock such as rough lemon (*Citrus jambheri*), or in plantings on resistant rootstock where the graft union is at or below the soil surface exposing scion tissues to the pathogen. If the disease is not checked in time the entire tree may be destroyed. The brown rot of fruits occurs in orchards causing fruit drop and in storage it causes post-harvest decay of fruits.

Symptoms

Phytophthora spp. can cause damping off of newly germinated seedlings of *Citrus* spp. Typical symptoms result when soil- or seed-borne fungus penetrates the stem just above the soil line and causes the seedlings to topple. Seed rot and pre-emergence damping off may also occur. There is rapid killing when there is abundant soil moisture and temperatures are favourable for the fungus. Once the leaves have emerged the seedlings become resistant. These symptoms are similar to damping off of any plant caused by *Pythium* or *Rhizoctonia* (205).

Foot rot and gummosis are the most serious diseases of citrus caused by *Phytophthora* spp. Primary infection by *Phytophthora* normally occurs on the bark at the base of the trunk near the ground level, producing lesions on the trunk and the crown roots. It spreads around the trunk girdling it and killing the tree. The bark and the wood both are affected. The infection can spread upward and down the roots, often causing fibrous root rot. The root damage is especially serious on susceptible rootstock in nurseries. Fibrous root rot may occur even in bearing trees where the

root damage causes tree decline and yield losses (178). In some tolerant rootstocks the fibrous root rot does not affect the fruit yield.

The main symptom of gummosis is oozing of gum from the affected parts on the trunk. Infected bark remains firm with small longitudinal cracks through which abundant amber-coloured gum exudation occurs. Citrus gum is water soluble. During rainy season the gum is washed down or gets mixed with soil near the ground level hence this symptom may not be clear. During summer gum deposits dry and stick to the bark making the symptom of gummosis very clear.

The root rot (69) and fibrous root rot symptoms are not seen in the early stages of the disease. However, the root rot destroys a major portion of the root system before well-marked symptoms are seen on the aerial parts. The effect of trunk and root infection is ultimately drying up of the tree. Before death the tree flowers profusely but fruits are small and drop before maturity.

Leaves of the affected trees show symptoms of nutritional deficiency. The veins turn yellow and there is premature leaf fall. In mandarin oranges, infection of leaves by *P. palmivora* is common in heavy rainfall areas. Water-soaked spots appear on the lower leaves and by the time the spots spread over the entire lamina the leaf drops. This usually results in heavy defoliation. The infection reaches the unripe, ripening and ripe fruits and produces water-soaked spots on the skin. This is brown rot of fruits (73, 207). The infection of fruits can occur directly when water splashed inoculum reaches the fruits near ground level. All the fruits on the tree may gradually become affected under humid conditions. Such fruits become soft and white fungus growth develops on the skin. Ultimately the fruit drops. The fungus continues to grow on fallen fruits.

The fruits which do not show symptoms and are still on the tree are harvested and packed. If fruits are untreated, the brown rot spreads from fruit to fruit by contact (112). In a few days of storage these fruits have a characteristic pungent, rancid odour. Brown rot epidemics are usually restricted to areas where heavy rainfall coincides with the early stages of fruit maturity (236). All cultivars are affected especially the lemons.

The causal organisms

Three species of *Phytophthora*, viz., *P. palmivora, P. parasitica* (*P. nicotianae* var. *parasitica*) and *P. citrophthora* attack citrus. In south India the main cause of gummosis and root rot is recognized as *P. palmivora* (170) although *P. parasitica* is also common as incitant of fruit rot in Karnataka (180). In other parts of India also *P. parasitica* was reportedly associated with gummosis. In the USA *P. parasitica* and *P. citrophthora* are the main pathogens of root rot of citrus. In addition, *P. hibernalis* and *P. syringae*

attack citrus fruits to a limited extent in areas with cool moist weather. *P. citricola* is reported to attack citrus in some tropical areas (21).

Phytophthora nicotianae B. de Haan var. *parasitica* (Dastur) Waterhouse

The hyphae are tough, irregularly wide upto 9 microns, but without typical hyphal swellings. Sporangiophores are more slender than the mycelial hyphae, irregularly or sympodially branched, the sympodia being close in moist air. Sporangia are papillate, and occasionally have more than one apex. They are broadly ovoid, ellipsoid, obpyriform to spherical, not noticeably narrowed at the apex, occasionally lateral or intercalary. They measure 38-50 × 30-40 (av. 40 x 38) μm and are deciduous with very short (2-5 microns) pedicel. Oogonia are usually produced in single cultures though often very scarcely or not until some weeks, but readily produced in dual cultures with opposite strains (the A_1 and A_2 mating types). Antheridia are amphigynous, spherical or oval, and 12-16 × 18 μm in size. The oogonial diameter is usually 22-29 μm, rarely 31 μm. Oogonia become rough, thick walled and yellowish brown with age. Oospores are markedly aplerotic and 18-20 (sometimes 20-25) μm in diameter. The oospore wall is about 2 μm thick (232). Chlamydospores are abundantly produced. They are less than 25 to 60 μm in diameter, with 3-4 μm thick wall. Minimum, optimum and maximum temperatures for growth are 10°C, 30-32°C and 47°C, respectively. *P. parasitica* has a broad host range. Virulence of isolates from tomato and other non-citrus hosts towards citrus is low while all isolates of the species are pathogenic on tomato (133).

Phytophthora citrophthora (Smith and Smith) Leonian

Hyphae are fairly coarse and upto 7 μm wide. Sporangiophores are delicate, short, scarcely widening at the base of the sporangium, irregularly branched and with a swelling at the point of branching. Sporangia are rather scanty on some agar media and very variable in shape and size in water; often with more than 1 apices and papilla having 5 μm deep apical thickening. Sporangia are deciduous with a 10-12 μm long pedicel. The average size of sporangia is 40-45 × 27, often 50-55 × 30 or even up to 90 × 60 μm. Chlamydospores may be abundant, few or absent. Most isolates do not form chlamydospores. Their average diameter is 28 μm with 1.5-2 μm thick wall. Sexual reproduction is not seen (233). Minimum, optimum and maximum temperatures for growth are 5°C, 24-28°C and 32°C, respectively.

Phytophthora palmivora (Butler) Butler

The mycelium is intercellular with haustoria. Hyphae are large and

often swollen at regular intervals. They are upto 7 μm wide. Sporangiophores are simple or branched with inverted pear shaped, rarely round and always terminal sporangia which measure 38-72 × 33-42 (av. 50 × 35) μm. Zoospores are large, 8-10 μm in diameter when encysted. Oospores are spherical, 35-45 μm in diameter with 4 μm thick wall. They produce secondary sporangium on germination.

Disease cycle

P. parasitica is a parasite but a poor saprophyte in soil. It causes noticeable yield losses only when high populations of the fungus are present in the soil. Tsao (215) and Tsao and Bricker (218, 219) had carried out extensive studies on the behaviour of the fungus in soil. The growth in soil is restricted due to antagonism including intense colonization of hyphae by bacteria leading to rapid lysis and breakdown of cytoplasm (125, 215). The entire mycelium is soon converted into oospores and chlamydospores which serve as survival structures. Oospores occur in low numbers in soil and probably are resistant to desiccation and cold temperature. The formation of oospore and perpetuation of the fungus through these structures is governed by the presence of mating types in the plant population (181, 182). The oospores mature more slowly than chlamydospores but once matured they germinate in response to nutrients from roots.

Chlamydospores are common and the most important source of soil survival of *P. parasitica* (197). These structures are commonly formed when soil moisture is limiting, conditions are cool or where the host roots are not actively growing and producing susceptible tissues. Formation of chlamydospores can also be stimulated by poor aeration and high carbon dioxide concentration in the soil atmosphere (213). They can survive in soil for several months under unfavourable conditions. In the presence of host roots, nutrients, aeration, optimum temperature and moisture these resting structures germinate by a germ tube and by sporangia which liberate motile zoospores. Germination of chlamydospores is optimum in well-aerated, moist environments when temperatures are favourable for root growth. Root exudates promote their germination (60). Chlamydospores cause more uniform infection than zoospores (90).

Kuch and Khew (115) noticed maximum populations of propagules of *P. palmivora* at 0.5-15 cm depth of soil and very low populations at 30-45 cm. Optimum soil moisture for survival of propagules was 25-45 per cent WHC at pH 6.5-7.0. The survival could be for upto 18 months in natural soil. Sporangia survive and remain infective after passage through the alimentary canals of two snail species. Ingestion of oospores of *P. palmivora* by garden snails facilititates their germination (179).

P. citrophthora grows best at 24°-28°C and *P. parasitica* at 28°-32°C. Abundant production of sporangia of *P. citrophthora* occurs at 20° while that of *P. parasitica* at 30°C. Maximum recovery of *P. citrophthora* from rootlets in naturally infested soil is obtained at 15°-20°C and that of *P. parasitica* at 15°-30°C (135-137). Thus, in citrus areas where temperatures are low *P. citrophthora* is more dangerous than *P. parasitica*. A temperature of 24°C after infection favours development of *P. citrophthora* in roots.

The number of propagules rapidly increases immediately after irrigation in irrigated orchards or nurseries. Lutz and Menge (124) have reported that in long interval furrow irrigation the initial population of 17 propagules/g soil increased to 77 propagules/g soil two days after irrigation. According to them very low numbers of oospores of *P. parasitica* are found in the soil throughout the year. Low levels of dark coloured multi-papillate sporangia were also consistently found by them in soil. Agostini, *et al.* (4) have stated that conditions which allow abundant immature fibrous root development, a highly susceptible rootstock, and favourable soil temperature and moisture promote development of root rot and, consequently, high propagule density of *P. parasitica* in soil.

In addition to oospores and chlamydospores in the soil as the main survival structures for initiating primary infection. *P. parasitica* also survives on fallen fruits, twigs, leaves, and in cracks on the standing trees. The main source for the spread of these pathogens in Nagpur mandarin orchards in central India is reported to be infested nurseries. More than 20% nursery plants die due to Phytophthora diseases and almost all nurseries are infested (149).

When chlamydospores and oospores germinate in the presence of nutrients, optimal soil moisture and aeration they form sporangia which liberate zoospores. Zoospore release is optimal in saturated soils. Nutrient depletion stimulates sporangia production (175, 215, 216). Diurnal temperature changes in soil may serve to synchronize the release of zoospores. According to Duniway (60) the motile zoospores swim to short distances (cm) or are carried by soil water to long distances (meters). Dispersal of sporangia and zoospores is by wind, raindrop splashes, irrigation water, and even insects. Zoospores are attracted to roots by root exudates (101, 220, 221), especially when roots are damaged but living (107, 108). On the root surface they encyst, germinate and cause infection by penetrating the cortex. Citrus tissue is more susceptible during the periods of the year when trees are actively growing than during the months when trees are dormant (132).

Heavy or fine textured soils where drainage is impeded, high soil moisture, pH of 5.4-7.5 and a temperature of 24°C are conducive to disease development. Soil moisture is the most important plant-soil environmental

factor that affects development of Phytophthora root rot of citrus. The host is pre-disposed to infection when roots are stressed or damaged in saturated or dry soil. Long periods of soil saturation are required for *P. parasitica* to be an effective root decay agent (217). According to Stolzy *et al.* (200) the length of saturation time is more important than the frequency of saturation. The frequency and duration of irrigation can also influence the activity of the fungus and pre-disposition of roots to rot (74). In sandy loam soils the greatest destruction of feeder roots occurs in irrigation furrows where saturated conditions favour zoospore production and movement. Roots outside the furrow often remain healthy (92). If soils are saturated during irrigation, zoospores are released and can infect roots to form more sporangia. When soils do not dry sufficiently between irrigations, sporangia survive until the next irrigation and again release zoospores. Soils with drainage restricted by hardpans or clay layers or those with shallow water table that temporarily rises into the root zone provide ideal conditions for fibrous root rot and build up of Phytophthora propagules.

Availability of oxygen in soil atmosphere is closely related to soil moisture because pore space for air is reduced in saturated soil. When roots are subjected to low oxygen conditions they are damaged by reduced forms of minerals and by toxic metabolites of microorganisms on the root surface. Root regeneration is restricted, new roots are not formed and root exudation increases under flooded conditions. Thus, in presence of *Phytophthora,* reduced oxygen level in soil causes greater root decay (200).

Low grafting and nearness of bud union to soil line increase chances of infection from soil-borne inoculum. Populations of the pathogens around roots in soil are increased where, in addition to the favourable environments, there are abundant immature fibrous roots and a highly susceptible rootstock thus promoting development of root rot. Around resistant rootstock there is low density of the fungi in soil. Most rootstock are at least moderately tolerant to *Phytophthora* (209) but their susceptibility varies (92, 166). Although mechanisms of resistance are not clearly understood, it is presumed that coumarin phytoalexins in infected roots play some role (1).

Microbial antagonism in the suppression of *Phytophthora* spp. in soil, rhizosphere or in the infection court is reported (124). Processes of parasitism, predation, and competition may all be operating alone or together in reducing inoculum. The reason for rapid loss of *Phytophthora parasitica* mycelium in soil is attributed to lysis induced by intense bacterial colonization of hyphae. Specific strains of *Streptomyces* and fluorescent pseudomonads occur in soil that cause hyphal lysis of *P. citrophthora* (22).

Vesicular-arbuscular mycorrhizae have been implicated in microbial antagonism of *P. parasitica* in citrus but increased tolerance of mycorrhizal

plants to root rot is probably due to improved host nutrition, mainly phosphorus (53, 91, 93, 94).

Management

A number of preventive measures can be recommended to reduce primary infection. The site for citrus orchards should be well drained land. Resistant rootstock such as Khatta and trifoliate orange (*Poncirus trifoliata*) may be used in areas where gummosis and virus diseases of citrus are serious problems (92, 166). The bud union should be about 30-45 cm above the base and at the time of planting care should be taken to keep the bud union well above the soil line. The irrigation system should be planned in such a way that water from below one tree does not flow to the other trees. Every year the trunk should be painted with Bordeaux paste up to a height of about 70 cm. If the soil is known to contain *Phytophthora* the walls of new pits should be dusted with a mixture of zinc sulphate, copper sulphate and lime (5 : 1 : 4). Cleanliness of the orchard is very important. All infected fruits, leaves, twigs, etc. that have fallen should be collected and burnt.

During the summer and rains the orchard should be regularly sprayed with copper fungicides such as Bordeaux mixture (4 : 4 : 50 or 5 : 5 : 50) or copper oxychloride formulations such as Blitox-50. Soil drenching with 1000 ppm terrazole is reported to totally inhibit *P. palmivora* up to 2.5 cm depth of soil (183). In more recent chemical treatments, systemic fungicides have been used (52, 68, 207, 204) for soil drench and, sometimes, for trunk spray. Working with root rot and crown rot (*P. citrophthora* and *P. parasitica*), Matheron and Matejka (134) reported that metalaxyl, fosetyl-Al (Phosethyl-Al), and sodium tetrathiocarbonate (Enzone, which releases carbon disulphide in soil) reduced production of sporangia in soil by 90%. There were no lesions when infested soil was treated with 10 microgram/ml metalaxyl. A single application of metalaxyl or fosetyl-Al can provide protection to citrus from colonization by *P. citrophthora* or *P. parasitica* for 2-3 months (131). In Turkey, fosetyl-Al is reported to protect trees against gummosis for at leat one year (66). Enzone is more effective than metalaxyl in eradicating *Phytophthora* from host debris in soil. According to Sastry and Hegde (183) metalaxyl could penetrate and cause inhibition of inoculum up to a depth of 1.25 cm of soil. According to Naqvi (149) the density of fungal propagules in soil treated with Ridomil drench or Ridomil spray as well as drench is reduced by 69.1-72.8%. The population of the pathogens in relation to feeder roots density is also decreased. Drench and spray treatment with fosetyl-Al (Aliette) or Ridomil significantly increased the feeder root density. For economic control applications of fungicides should coincide with periods favourable for

pathogen activity and disease development. Extremes in temperature (35°C at the max. end) are not favourable for the pathogen and at such periods fungicide application is not required. Application of metalaxyl or fosetyl-Al is beneficial when threshold level of the fungus reaches 10-15 propagules per cubic centimeter of soil (*cf.* 4). In general, the chemical treatments are required to be done before rains start because the pathogen multiplies rapidly during rains.

Although chemical control of citrus feeder root rot in the field with fosetyl-Al and metalaxyl is effective, it also is expensive. Over the years, emphasis has shifted to the production of nursery trees free of *Phytophthora* spp. by preventive phytosanitary methods such as soil fumigation, treated irrigation water and sound hygiene. Such healthy nursery trees grow consistently better than infected nursery trees. However, nursery plants that are initially certified disease-free and planted in virgin soil eventually become infected by irrigation water sources which are frequently contaminated by *Phytophthora* spp. and nematodes. The contaminated irrigation water supplements existing *Phytophthora* and nematode populations in the soil, making chemical control more difficult. Use of bleaching powder (chlorine) in irrigation water for nursery beds can reduce *Phytophthora* spp. on the planting stock. Application of chlorine on a field scale was considered costly. Electrolytic method of chlorine generation makes the water treatment by chlorine cheap and makes it possible for field application. Chlorine kills the propagules of *Phytophthora parasitica, P. citrophthora* and *Fusarium* spp. Dipping roots of grafts for 6-10 min in water at 35°C or in 0.02% suspension of captan and soil fumigation with Vapam or Mylone had been routine practices in many citrus growing countries to reduce losses from gummosis and root rot.

An integrated chemical treatment for Phytophthora root rot and the citrus nematode, *Tylenchulus semipenetrans* which causes citrus decline, has been proposed by Le Roux *et al.* (120). They used metalaxyl or fosetyl-Al as fungicides and aldicarb (Temik) as nematicide. In 24 months treatments were given at 3 months interval with 5% metalaxyl granules as soil drench at the rate of 2 g a.i. per sq. metre of leaf canopy and 15% aldicarb at 4.5 g a.i. per sq. metre of leaf canopy. The treatments resulted in increased trunk diameter and canopy volume. After 32-44 months of commencement of treatment fruit yield was significantly increased.

Eradication of infection from standing trees is possible only if the disease is detected in the early stages. If infection is on thin branches they may be cut and burnt. In the early stages of root rot, affected roots may be removed and the soil drenched with a fungicide. On thick branches and trunks the infected portion may be removed by a sharp knife and the wound cleaned with 0.1% mercuric chloride or 1% potassium permanganate solution followed by application of Bordeaux paste on the wound.

Generally, the above mentioned chemical treatments reduce the chances of brown rot of fruit. Copper fungicides or captan applied prior to beginning of rains are usually quite effective. Pre-harvest application of systemic fungicides metalaxyl or fosetyl-Al to the canopy provides effective control of brown rot (43). Post-harvest disinfection of fruits with chlorine or sodium orthophenylphenate, recommended for canker affected fruits, can also be helpful. Hot water treatment of grapefruit (48°C, 3 min), lemon (52°C, 5-10 min) and orange (53°C, 5 min) has been reported (10). Hot water treatment of certain citrus fruits has limitations (108). Although lemons could routinely tolerate immersion in water at 46.1°-48.9°C for 4 min or longer without injury, release of rind oils leading to oleocellosis could occur if lemons were cold and turgid at the time of treatment (111). The immersion should, therefore, be delayed by 1-4 days after harvest to allow the rind to lose turgor. Without this conditioning the fruits can be injured even at 37.8°C. Using soap in post-treatment rinse of fruits entraps released oils and terpenoids to further reduce the chances of rind injury.

■ ANTHRACNOSE, DIE-BACK OR WITHER TIP

Anthracnose is one of the causes of citrus decline (6, 68, 166). The disease is especially serious on orange, grapefruit and lemon trees. It affects all mature, weakened or injured aboveground plant parts, including leaves, twigs and fruits. Anthracnose may occur on trees of any size, in the nursery or in the orchard, but it rarely develops on vigorously growing trees. It is common on trees that are weakened or injured due to inadequate fertilization, lack of water, low temperature, insect attack, etc.

Symptoms

Wither-tip and die-back: The main symptoms of this condition are falling of terminal leaves on the shoots and drying of the shoots (69). The drying starts from the tip and progresses backward. The dry portion is straw or ash-coloured. A large number of black dot-like structures representing acervuli of the fungus appear on the ash-coloured portion. These symptoms are more pronounced during the summer and rains than during the winter.

Anthracnose: When the symptoms due to necrosis appear on leaves, fruits and twigs, they are called anthracnose. Reddish brown spots appear on the leaves which are disfigured. The central portion of these spots turns gray or ash-coloured and acervuli can develop on this portion. On the shoots, in addition to die-back, tissue necrosis may occur at any poin

Similar grey or black spots appear on the inflorescence stalk and flowers which shed. In fruit infections, reddish brown, circular and sunken spots are seen. They may be tiny specks or dark brown or black areas of 5-10 mm diameter. Usually they are only skin deep but in over-mature fruits they affect the flesh also. The spots become dry and hard and sometimes are dotted with small black acervuli that exude pinkish masses of spores in humid weather. These spots may appear on any part of the fruit surface but usually they start from the stem-end. Over-ripe fruits are particularly susceptible to anthracnose. When spores of the fungus are washed down from the twigs onto the fruits they germinate there and cause anthracnose russetting. The russetting appears as a large blotch or as a tear stain. Diseased leaves, flowers, apical parts of shoots and fruits drop down prematurely. In severe infections there may be considerable defoliation. In grapefruit (*Citrus paradisi*) a drop of fruits before ripening has been found during the rainy season. Usually such fruits do not show any spotting. But isolations from the stem-end tissue yield the fungus associated with anthracnose (184). Infected tissues show reduced amount of different sugars. Infection affects the amino acid content of the tissue also. Some amino acids are decreased. This varies with the host species.

Post-bloom fruit drop: The post-bloom fruit drop is caused by a strain of the same fungus that causes typical die-back and anthracnose (67). Peach to orange-coloured necrotic spots are formed on flower petals. Under favourable conditions entire flowers and clusters are invaded resulting in blossom blight (211). Abundant acervuli are produced on the surface of blighted flowers. After flower infection fruitlets drop, but the buttons composed of peduncle, floral disc, calyx and nectaries remain (210, 213). These symptoms are same as those described above under anthracnose and for premature fruit drop (188).

The causal organism

Fawcett (69) had recorded *Colletotrichum gloeosporioides*, *Gloeosporium limetticolum* and *Gloeosporium foliicolum* as pathogenic and causing anthracnose of citrus. *C. gloeosporioides*, described under mango anthracnose is most common. There may be several races with varying levels of pathogenicity within each species of the anthracnose fungus.

Disease cycle

The anthracnose fungus is favoured by high temperature and humid or moist weather. A relative humidity of 95% is optimum for growth of the fungus. The conidia are released only when the acervuli are wet and are generally spread by splashing and blowing rain or by coming in contact

with insects, other animals, tools, etc. (3, 209). They germinate only in the presence of free water. The germ tubes produce appressoria and penetration pegs enter the host directly. In the beginning the hyphae may grow rapidly, intercellularly and intracellularly, but cause little or no visible discolouration or other symptoms of disturbance. Then, quite suddenly, especially when fruits begin to ripen, the fungus becomes more aggressive and symptoms appear.

Some workers believe that die-back symptoms are not caused directly by fungi. Nutritional deficiencies and attack of viruses and citrus greening bacterium first cause the die-back and later the fungus grows saprophytically on the dead or weakened parts. *C. gloeosporioides* produces non-specific toxins also which are supposed to induce die-back (185).

Management

The disease has a close relationship with poor growth of the trees. Priority should be given to measures that help in vigorous tree growth. Presence of hard pan below the root zone, accumulation of salts near the roots, and general low fertility level of the soil should be taken care of before planting and during growth of the trees. The diseased twigs should be pruned before the warm rainy season starts. Fallen leaves, twigs, and fruits should also be collected and burnt.

Spray of copper fungicides during the summer and rainy season had been recommended in the past for the control of citrus anthracnose. After pruning the cut ends of the branches should be protected by a fungicidal paste. Fungicides recommended against the anthracnose disease include Bordeaux mixture, copper oxychloride, benomyl, zineb, maneb, mancozeb, chlorothalonil, captafol and folpet. Benomyl and captafol alone or in various combinations have proved the most effective fungicides for the control of post-bloom fruit drop. One to four applications during the bloom period are generally recommended. The number, rather than timing, of applications during the bloom period are important. When blossom blight incidence is low only one application of benomyl or captafol at mid-bloom or two applications, one at early bloom and one at mid-bloom control the blossom blight. In a severe incidence, weekly or 10-day schedules provide high degree of control of blossom blight and button formation (210). In acid lime (*C. aurantifolia*) three sprays of 1% Bordeaux mixture starting from the first week of July, at 21 days intervals, have been found most effective in India. Carbendazim (Bavistin) at 0.1% also gives effective control of die-back.

ARMILLARIA ROOT ROT

Armillaria root rot is of worldwide distribution and affects hundreds of species of fruit trees including citrus, apple, pear and grapevine both in the temperate and tropical regions. It has been described under different names such as 'shoe string root rot', 'mushroom root rot', 'crown rot' and 'oak root fungus disease' (5). The pathogen, *Armillaria mellea*, is a common fungus in forest soils. The disease is common in orchards or vineyards planted in recently cleared forest land. The losses are inconspicuous, appearing as slow decline and death of occasional trees, with greater number of trees dying from the disease during periods of moisture stress and after leaf fall (5).

Symptoms

The above ground symptoms on the tree are similar to those caused by other root diseases. These include loss of tree vigour, reduced growth, smaller, yellowish leaves, die-back of twigs and branches, and gradual or sudden death of the tree, especially when the crown or collar is affected. The disease starts from root infection. The fungus continues to grow underground on the roots but aboveground symptoms appear late. Sometimes, no symptoms are seen but the tree suddenly wilts. Occasionally, only the top of the tree dries or branches on one side dry and rest of the foliage remains healthy. In the orchard, if leaves on the tree are turning yellow and are few and the tree shows poor growth Armillaria root rot can be suspected (55). Initially, scattered trees show symptoms but later they may be found in circular patches.

Specific symptoms can be seen at the foot of the tree or on the roots. The bark shows decayed areas on the collar and the roots. Infected roots are swollen and as the fungus grows they are destroyed. Below the bark, radiating, white, velvety masses of fungus mycelium are seen. This mycelial web soon destroys the bark and wood and grows out on the surface or in surrounding soil as hard, black or deep pink ropes (rhizomorphs). The shape of these growing hyphal strands is somewhat similar to shoe laces. At the apical portion of this growth the fungus forms mushrooms or carpophores. These fruiting bodies of the fungus can be seen on the lower trunk or around the trunk on the soil. Soon these fruiting structures break and fall down on the ground. By this time most of the roots are destroyed. As a result of infection, the trees either suddenly wilt or signs of slow decline appear. This depends on the number of roots destroyed by the fungus.

The causal organism

Armillaria mellea (Vahl ex. Fr.) P. Kaarst (syn. *Armillariella mellea*) belongs to the mushroom group of fungi (Agaricales). The mycelium is white, velvety, and radiating in a fan-like manner. The individual hyphae are thin and incapable of penetrating the host tissue. They aggregate into thick strands of hyphae (rhizomorphs) which are 1-3 mm thick and only these bundles of hyphae can cause infection of the host. They consist of a compact outer layer (sheath) of black mycelium and a core of white or colourless mycelium. Often the rhizomorphs can form branched network on the roots, under the bark, or in severely decayed wood. Some strands spread out in to the soil surrounding the roots. These rhizomorphs enter the roots through medullary rays. At the base of dead or drying trees the apical portion of the rhizomorphs develops the mushroom or the basidiocarp (the fruiting body of the fungus). These could be few or few to many, honey coloured, speckled and 9 or more cm tall with a cap (pileus) of 5-15 cm diameter. The lower surface of the pileus contains numerous gills in which basidiospores are formed. The basidia are elongate clavate, 34-47 × 5-9 μm in size and bear four sterigmata which are up to 6 μm long. The basidiospores, borne on sterigmata, are short, ovoid to ellipsoid, and measure 7-12 × 5-7.5 μm. These basidiospores cannot infect living tissues of the host. They fall on dead wood, stumps, or injured surface and develop the mycelium and rhizomorphs. The latter grow and cause infection of roots.

Disease cycle

Disease severity in Armillaria root rot is associated with relative amount of residual woody debris, especially roots, from trees present when the land is converted to new plantations. In orchards where the disease has established, the fungus mainly survives through rhizomorphs in soil or as mycelium and rhizomorphs in infected roots and trunk in which the survival for a minimum of 6 years is reported (80). Basidiospores have little direct role in survival. The principal method of tree to tree spread of the fungus is through rhizomorphs or direct root contact. Rhizomorphs grow from roots of infected trees or from decaying roots or stumps through the soil to roots of adjacent healthy trees. The rhizomorphs must remain connected to a food base such as an infected root or stump in order to grow and to infect other roots. Nutrients are transported from the food base to the rhizomorph tip but not the reverse (174). However, from experimental evidence Morrison (144) suggested that rhizomorphs absorb nutrients from the soil. Soil rich in organic matter supplies more nutrients. Thus, soil is the principal source of nutrients for rhizomorphs. In countries

like Zambia and Zimbabwe, where rhizomorphs are not formed (203) due to toxic materials in the soil, spread of *Armillaria* is only by root contact. Pieces of rhizomorphs can also be dispersed by various cultural operations in the orchard. The pathogen produces antibiotics that protect it from antagonistic fungi. But if the fungus is weakened antibiotic production is less and the mycelium can be destroyed by antagonists like *Trichoderma viride* (230).

The pathogen is active in warm, moist soil and is inhibited by cool and dry soil (176). The optimum temperature for growth of the fungus is 21°-25°C but it causes maximum infection of host roots at 10°-18°C when the growth of roots is slow (16). The optimum for growth of citrus roots is 17°-31°C, similarly for peach and apricot it is 10°-17°C while the optimum for root damage in these hosts is 15°-25°C. This suggests that the severity of the disease depends on adverse effect of temperature or other factors on the host. Host defoliation due to attack of gypsy moth is reported to predispose the trees to infection. Rhizomorphs continue to elongate while covered with a water film, but without the water film, more oxygen reaches the outer cells of the rhizomorph, pigmentation occurs and growth is checked.

Management

Eradication of *Armillaria* from orchards is a difficult and time consuming operation. Losses can be reduced by removing the substrates such as stumps and decayed roots and avoiding or delaying new plantations for several years on land that has been recently cleared from forest trees, especially oak trees. The badly affected trees should be dug out along with all possible root vestiges. A trench around the place of the removed tree should be dug and the area enclosed by the trench should be fumigated with carbon disulphide for several times at 6 months interval until all vestiges of roots have decayed (16). Trenching around lightly infected trees and applying fumigants in the trench also prevent spread of rhizomorphs to healthy roots. Dig and fumigate has been the most commonly adopted practice in citrus orchards in USA.

Soil fumigation with carbon disulphide had been shown to kill *Armillaria* as early as 1914 (160). By 1951, it was noticed that while high dosages of the fumigant killed the fungus in 2-3 days, lower or moderate dosages killed it only after 30-59 days in field soil and not at all in sterilized soil. *Trichoderma viride*, the antagonist was thought to attack *Armillaria* only when the latter was weakened by fumigation (*cf.* 45). Methyl bromide has similar effect as carbon disulphide. Compared to the pathogen, the antagonist has a high tolerance to the fumigant. Heating the soil to 33°C for 7 days with aerated steam also weakened the pathogen (146, 147).

Temperature of 36°C for 7 days or 43°C for 2 hours kills the pathogen. Since living citrus and peach roots are not injured by such temperatures, heat treatment of planted trees is a potential measure for protecting valuable specimen trees.

■ POST-HARVEST DECAY OF CITRUS FRUITS

Post-harvest rot of citrus fruits, particularly mandarins and oranges, may result from infections of the fruit on the tree such as *Colletotrichum gloeosporioides* causing anthracnose (24), *Phytophthora* spp. causing gummosis root rot and brown rot of fruits, *Phomopsis citri* causing melanose, *Diplodia natelensis* and *Lasiodiplodia* (*Botryodiplodia*) *theobromae* causing die-back. In these cases the pathogens may occur as microscopic surface contaminants of the fruit skin, or in soil and debris on the skin or stem tissues. The infection remains latent and symptomless and the fruit rot develops later when physiology of the fruit permits growth of the pathogen. The post-harvest decay can also result from infection of fruits during harvesting, transport and other handling operations due to spores of fungi (*Penicillium*, *Aspergillus*, *Rhizopus*, and *Alternaria*) being present in the atmosphere or on the orchard soil, in the stores and in the containers. The rot of fruits after harvest by one or more of these fungi is responsible for 10-15 % or even up to 30 % loss during transit, storage and in the market. There are many reviews of post-harvest fruit pathology (62, 63, 64, 159, 162).

Blue, Green, and Black Mold Rots

Various species of *Penicillium* cause the blue mold rot (*P. italicum*), and green mold rot (*P. expansum* and *P. digitatum*). These are the most common and the most destructive post-harvest diseases affecting all kinds of citrus fruits throughout the world in addition to apples, pears, quinces and grapes. Although some infection of citrus fruits can occur in the orchard (Penicillium mold of oranges), these molds are essentially post-harvest diseases and account for up to 90 % of the total loss due to various types of rot in transit, storage and in the market.

Penicillium rot at first appears as soft, watery, slightly discoloured spots of varying size on any part of the fruit. The spots are at first rather shallow but quickly become deeper and at room temperature most of the fruit or the whole fruit decays in just a few days. Soon after the decay starts a white mold begins to grow on the skin surface near the centre of the spot. Later, the growth starts producing spores. The sporulating growth has a blue, bluish green, or olive green colour and is usually surrounded by a narrow or wide band of white mycelium with a band of water-

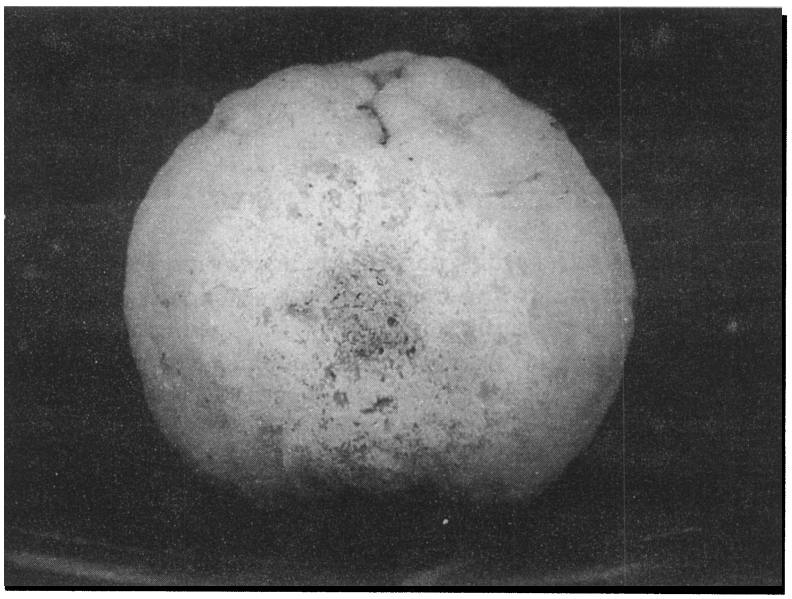

Fig. 1. Penicillium rot of mandarin orange.

soaked tissue ahead of the mycelium. The surface growth of the fungus develops on spots of any size as long as the air is moist and warm. In cool, dry air the surface growth is rare even when the fruits are totally decayed. Under storage conditions, small tufts of spore bearing hyphal branches appear on the surface of the spots. The quantity of spores produced is enormous and these spores get in to the atmosphere, landing on healthy fruits and spreading the rot. Decaying fruits have a musty odour and in very dry conditions may shrink and mummify while under moist conditions, when secondary fungi and yeasts also enter the fruit, it is reduced to a wet, soft mass.

Penicillium is the imperfect (conidial) stage of the Ascomycetous fungus *Talaromyces* (Eurotiaceae in Plectomycetes of Ascomycotina). Only the conidial stage is common in storage rots. The hyphae are branched and septate. The conidiophores arise in clusters (tufts) forming definite fascicles (coremia). They are smooth, 600-700 μm long in *P. expansum*, often intertwined and asymmetrically branched. The final branches bear conidia in tangled chains. These chains may be up to 200 μm long. The conidia are elliptical and measure 3-3.5 μm in diameter. Their wall is smooth and in mass gives the characteristic colour of the mold.

Penicillium spp. are saprophytes and continue growing and producing spores on decaying organic matter. The spores are present in the atmosphere. These fungi enter the fruit tissue through breaks in the skin

(bruises and wounds) and through the lenticels. However, fruit-to-fruit spread during transit can occur without skin injury.

Generally, for infection, the spores must get dead tissue which the bruises and wounds on the fruit skin provide. Once lodged on the wounds the fungus feeds on the dead cells, produces macerating enzymes which kill more cells on which the mycelium advances. The water-soaked band ahead of the growing mycelium represents the area of enzymic action. Although most of the rot is seen during storage and at the market and there is spread during contact between fruits (11), the occurrence of these molds is greater when (i) the fruits are harvested and handled during wet, humid weather than in cool and dry weather, (ii) when fruits are delayed in going in to the storage, (iii) when fruits are injured during handling, (iv) when they are stored for long, and (v) when they are held at warm temperatures after removal from storage. The rot is favoured by high storage temperatures but the fungi continue growing even at temperatures near freezing. The green mold fungus (*P. digitatum*) can cause rot even at 0°-5°C. Some species produce ethylene which diffuses in to the containers and hastens ripening of fruits pre-disposing them to secondary infection and rot. Some species produce mycotoxins such as patulin which cause damage to intestines, kidney and liver. They can affect the nervous system and may also cause cancer.

The black mold rot of citrus fruits caused by *Aspergillus niger* also produces similar symptoms but the surface growth looks black. This fungus produces chains of conidia on small stalks on a vesicle formed at the tip of the conidiophores. In acid lime (*Citrus aurantifolia*) this species is reported to cause 10-15 % loss during transit and storage.

Stem-end Rots

In this type of decay, the rot generally starts from the stem-end of the fruit. The rind becomes soft and lighter or brown colored. If the fruit is cut open, the rot is seen growing in to the core and margins of juice sacs. Later, the entire fruit becomes soft and internal tissues totally rot.

Several fungi are reported to cause stem-end rot in citrus. Most important is *Phomopsis citri* (teleomorph *Diaporthe citri*) which incites the disease known as melanose in the orchards. This fungus causes small, round, black spots on young leaves. The spots have yellow margins. Later, the centre of the spots is raised and becomes rough and brown. Similar lesions are formed on twigs and fruits also. The other fungi which have been reported to cause stem-end rot are *Diplodia natalensis* (36) and *Botryodiplodia* (*Lasiodiplodia*) *theobromae* (199). These two species are described under post-harvest diseases of mango. In *Phomopsis citri* the conidial stage is common on green parts of the tree. Perithecial stage

(*Diaporthe citri*) develops on twigs lying on the ground. Conidia are produced in pycnidia which are scattered or clustered, at first immersed, becoming erumpent, black, conical to lenticular, ostiolate and up to 600 μm in size. Conidiophores are phialidic, hyaline, simple and cylindrical to obclavate. Conidia are hyaline, 1-celled, fusiform to ellipsoidal and 6-10 × 2-3 μm in size. Another type of spores (stylospores) may also be produced. These are hyaline, filiform, 1-celled, curved and often strongly hooked, and measure 20-30 × 0.5-1.0 μm. In its perithecial stage the fungus produces cylindrical to clavate asci containing 8 ascospores which are bi-celled, constricted at the septum, and germinate by 1-2 germ tubes.

In citrus, infection by *Lasiodiplodia* and *Phomopsis* via the floral parts is reported. These fungi colonize the injured peduncle and pedicel of citrus fruits, remain quiescent (latent) under the sepals or in the button (calyx + disc), and enter the fruit only after abscission occurs. Thus, chemically induced delay in abscission reduces stem-end rot. Post-harvest treatment of fruits with 2,4-D prevents abscission of the button. Uninjured fruit is infected by *L. theobromae* only through the exposed surface of the pedicel and pedicel scar. The fungus cannot enter the intact rind even if the fruit is fully ripe.

Alternaria Black Rots

Different species of *Alternaria* cause rot of a variety of fruits before or after harvest. Among citrus fruits, mandarins (*C. reticulata*) and sweet oranges (*C. sinensis*) are particularly susceptible to these rots. Two types of symptom development are seen. In one, spots are seen on the rind of the fruit and rot proceeds to different depths in the fruit. In the other, the fruit outwardly looks healthy without blemishes on the skin but inside the core and/or juice sacs show a black rot.

Fawcett (69) had mentioned the Alternaria rot of oranges and lemons caused by *Alternaria citri* Ellis and Pierce. Subsequently, Keily (106) described a brown spot disease of mandarins caused by *A. citri* in Australia. In India, Alternaria rot of sweet oranges and lemons caused by *A. citri* was reported in 1967 (2). A black core rot of mandarins in India was attributed to *A. tenuis* Auct (189). Logrieco *et al.* (121) have reported a similar rot of mandarins caused by *A. alternata* (Fr.:Fr.) Keissel from Italy.

In the infection of *A. citri*, brown spots appear on the rind, especially at the stem-end. These lesions increase in size to 2-3 cm. Dark blue or black growth of the fungus develops on the spots. The decay grows in to the fruit and destroys the tissues up to the core. In the core rot of mandarins caused by *A. tenuis* (189), initially the fruit looks healthy from outside. The fungal growth is seen only in the core where the empty space is filled with dark bluish growth of the fungus. Sometimes, there is

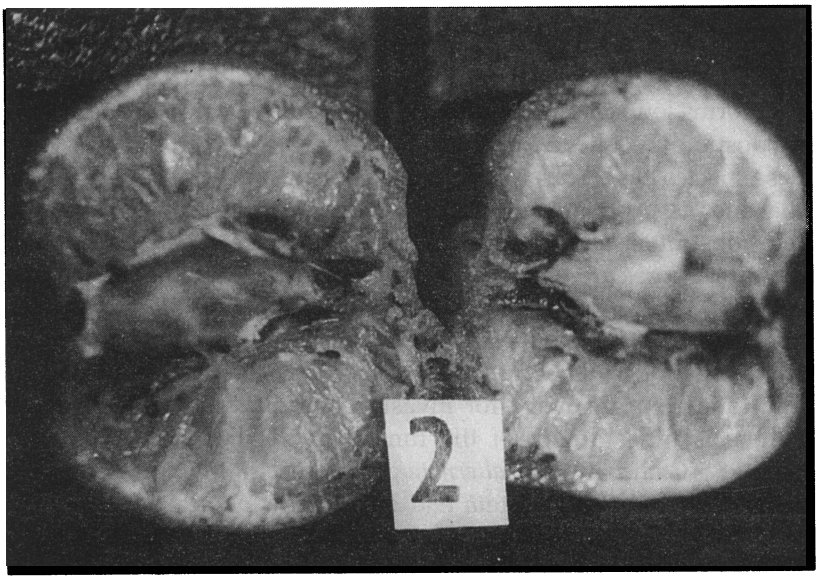

Fig. 2. Alternaria core rot of orange.

browning of the skin at the stylar end. When the fruit is opened, the rot appears to grow from the stylar-end or stem-end towards the core. The decay gradually spreads and destroys the juice sacs. After prolonged storage, when the internal tissues are destroyed, the decay reaches the rind and produces brown spots. In the rot of mandarins caused by *A. alternata* the symptoms are more or less similar to as described above but the rot is more in and around the juice sacs than in the core. In the first stage of the disease, fruits do not show any symptom on the outside but later the surface turns dark starting from the stem-end. In the advanced stage of the disease, the fruits generally fall down from the tree. In this case two strains of the fungus are involved. One produces a dark coloured growth and the other produces a grey coloured growth. In the dark strain there is heavy sporulation. The species is very similar to *A. citri* and produces several mycotoxins (121).

Alternaria belongs to Dematiaceae of Hyphomycetes in Deuteromycotina. In *A. citri* conidiophores are simple or branched, straight or flexuous, septate, light or olivaceous brown, up to 3-4 μm thick and with a terminal or 1-2 lateral scars. Conidia are solitary or in simple or branched chains of 2-7, straight or slightly curved, commonly obclavate or ovoid, pale- to mid- or sometimes dark-brown or olivaceous brown, with up to 9 transverse and numerous longitudinal or oblique septa. They are constricted at the septa. The conidia measure 8-60 (42) μm long including the beak when the latter is present and 6-24 (17) μm thick at

the broadest part. The beak is mostly 8 microns long and 2.5-4 μm thick, hyaline or pale brown. In *A. tenuis* the conidia are similar but measure 14-45 (34.5) × 10-15 (13.4) μm with a beak length of 1-12 (3.6) μm (189). *A. alternata* forms long chains of conidia with a longer beak. It also produces chlamydospore in culture.

These species have a large host range and can survive through resistant conidia and as mycelium on plant debris. *A. tenuis* can survive on a suitable substrate as mycelium for 12 years. Conidia can also survive for many years. Spores are dispersed by wind and rains and reach the fruits on the trees. Where symptoms first appear as spots on the fruit surface, the infection is through wounds during handling operations. In core rot, latent infection of fruits occurs on the tree. In core rot of mandarins the optimum temperature range for invasion of fruits is 25°-30°C (190). At 20°C there is little growth of the fungus in the fruit and there is no infection at 5°C and 36°C. *A. alternata* grows best at 28°C. The pathogens cause early infection of the stem end in the growing season and enter a latent or quiescent state in the styler end soon after the initial stages of infection are completed. After harvest fruits gradually lose their resistance as they ripen. Generally, the problem of Alternaria rots is with ripe or over-ripe fruits and when there are wounds on the skin or when the tissue is weakened.

Sour Rot

Sour rot caused by *Geotrichum candidum* Link: Pers (31) is reported on many fruits such as citrus, apples, plum and litchi and on many vegetables including tomato and beans. It is a major post-harvest disease throughout the world. In India, the disease was first described in 1978 (186).

Infected areas on the fruit appear water-soaked and soft and the skin is easily punctured in the affected area. The decay spreads very rapidly, at first mainly inside the fruit, and eventually invades the whole fruit. Later, the skin frequently cracks over the affected area revealing a white, cheesy or scum-like fungal growth. A compact, cream-coloured fungal growth develops on the surface also. The entire inside of the fruit becomes a sour-smelling, decayed watery mass. Fruit flies attracted to the decaying tissue carry the fungus to other fruits.

Conidia of the fungus are aseptate, hyaline, with a thick and smooth wall. They measure (on citrus) 6.6-12.8 × 3.4-5.3 μm (126). The fungus is widely present in the soil and in decaying fruits and vegetables. Infection of healthy fruits occurs during or after harvest through stem scars, skin cracks, cuts and punctures of various types and by contact with decaying fruits (105). Mature green fruits are resistant to infection but can be artificially infected if submerged in water for 24-36 hours before storage (44). Pericarp

of the fruit acts as a barrier against infection (122) hence only cuts and punctures or natural scars deep enough to allow entry into the endocarp favour infection. The disease develops rapidly on tree-ripe fruits (12, 61) especially if harvested when the rind is highly turgid and the fruits are kept in moisture-holding plastic bags or packages. Fruit ripeness, nutrition, pH, high water content in the rind, temperatures of 25°-30° C, and high atmospheric humidity favour infection and rapid decay. Low oxygen and high carbon dioxide environment stimulates growth of the fungus. Synergism of *G. candidum* and *P. digitatum* in infected citrus fruits is also reported (143).

CONTROL OF POST-HARVEST DECAY OF CITRUS FRUITS

The approaches to management of citrus decay can be divided into two parts: pre-harvest and post-harvest. The pre-harvest treatments involve management of such tree diseases as anthracnose, gummosis and brown rot which also cause post-harvest rot, and tree and orchard sanitation. In post-harvest treatments of the fruits, chemical, physical and biological control methods are involved. Integration of different approaches ensures better management of fruit rots.

1. Cultural and Sanitary Precautions

The preliminary precautions necessary for maintaining tree vigour involve proper selection of the site for planting trees, proper routine fertilization or manuring, and proper water management. These steps avoid tree stress and provide the right type of tissue strength to the fruits. The first step in sanitary precautions should be proper selection of planting stock. It should be free from infection or inoculum of any fungus, bacterial and virus or viroid disease, particularly anthracnose, root rot, and tristeza. Infected parts of the tree should be pruned off and all types of plant debris from the orchard floor should be removed and destroyed. The cut ends of the branches should be protected by a fungicidal paste and routine sprays of fungicides for control of tree diseases should follow the pruning operation.

2. Picking and Harvesting

Wet and cloudy weather is not suitable for harvest of fruits. Maximum care should be taken to avoid cuts and bruises on the fruits during packing and transporting them from the orchard. Some training of the workers in this regard is essential. The boxes or baskets for transport should be clean, preferably sanitized by some anti-microbial solution. In some diseases, a fungicidal spray just at or a week before harvest is

recommended (26). At the time of harvest fruits should not be over-ripe. All damaged, over-ripe, and soft fruits should be segregated in the orchard itself. Such fruits can be disposed off in the orchard or packed separately. The time gap between picking and storage should be minimum. A temperature of 10°C in store for short or long duration storage is considered ideal for avoiding most of the storage rots. In advanced economies, storage and transport under low oxygen (5%) or increased carbon dioxide levels (5-20%) have been used to suppress respiration of both the host and the pathogen thereby suppressing post-harvest rots. The results are further improved by the addition of 10% carbon monoxide.

3. Post-harvest Treatments of Fruits

This aspect of post-harvest fruit and vegetable pathology has been extensively investigated in many countries. Physical treatments (gamma radiation, heat removal or chilling, low temperature storage), chemical treatments (dips or sprays with fungicides and antibiotics) and biological protection (use of antagonists as fruit dip or spray) have been used.

Physical treatments affect both the pathogen and the host. Low temperature halts growth of the pathogen and also slows down the ripening of fruits. However, post-treatment susceptibility to fresh infections also occurs following heating, refrigeration and irradiation. Various types of radiation such as cathode rays, ultra violet, X-ray, and gamma ray have been tried and found to be highly fungicidal. Although all kinds of UV radiation (wave lengths above 280 NM) damage plant DNA and physiological activities, radiation below 280 NM at low dosages induce resistance in citrus, apples and peaches against post-harvest storage rots, and improve the shelf life of fruits (57, 122, 123). Gamma ray treatment (13) has been found better than others because of better penetrability. Gamma radiation stops growth of the pathogen by impairing mitotic cell division. It has been used against Penicillium rot in citrus. Irradiation is a costly process and its facility is not available everywhere. It is recommended only in serious disease problems.

It is always desirable to store fruits at the lowest temperature that does not harm them. For citrus fruits temperatures around 10°C are satisfactory. Many molds continue to grow even at lower temperatures but the decay is considerably slowed down till disposal in the market or until consumption in the homes. Disease development could be stopped by storage at temperatures above the maximum for growth of the pathogen. This is possible when the pathogen has a relatively low maximum temperature for growth such as *Monilinia* on pome and stone fruits. The citrus mold fungi have relatively high temperature maxima for growth. Storage above these temperatures may spoil the fruits. Brief exposure of fruits to heat through hot water is, therefore, recommended.

The temperature-time requirement for hot water treatment of citrus fruits (10) is given below:

Grapefruit	*Phytophthora*	48°C, 3 min dip
Lemon	*P. expansum* and *Phytophthora* sp.	52°C, 5-10 min dip
Oranges	*Diplodia, Phomopsis, Phytophthora*	53°C, 5 min dip

Hot water treatment of oranges causes poor degreening. Limitations in hot water treatment of lemons were mentioned with brown rot control measures.

4. Chemical Fruit Treatments

Field sprays of fungicides, especially before harvest, not only manage the associated diseases of leaves, twigs, stem and blossoms but also check incipient infections of developing fruits before harvest, which later cause decay of ripening or ripe fruits (*Diplodia, Phomopsis, Colletotrichum, Botryodiplodia*, etc.). These decay fungi can also be checked to some extent by post-harvest chemical treatments of fruits.

With pathogens which attack fruits during or after harvest, the decay can be controlled by use of chemicals to prevent infection and suppress development of the pathogens on the surface of the fruit. Most commonly used chemicals and commercial fungicides have been borax, diphenyl, sodium orthophenyl phenate, dichloran (DCNA), thiabendazole (28, 134), benomyl, thiophenate methyl, benzimidazole, captan, iprodione, vinclozolin, imazalil (27) and triforine. Some fungicides such as dichloran and biphenyl and acetaldehyde vapours, ammonia-emitting or nitrogen trichloride-forming chemicals are used as in-package fungistats impregnated in paper sheets during storage and transport.

The effectiveness of a fungicide in post-harvest treatment depends on the depth of inoculation, growth rate of the pathogen, susceptibility of the fruit to infection, temperature and humidity, and depth to which the inhibitory concentration of the fungicide can penetrate. *P. digitatum* on citrus fruits grows at a rather slower rate than *Rhizopus* on some other fruits. An effective fungicide will satisfactorily control decay by *Penicillium* if applied within 24-36 hours of infection at room temperatures. The fungicides that can penetrate the rind to some depth or are locally systemic are better than fungicides which have only surface action. Development of resistance to systemic fungicides and even some protectant fungicides is common among molds and may cause failure of the treatment. Therefore, precautions must be taken to include, additional, preferably broad spectrum, fungicides in the control programmes.

Borax was among the first chemical treatments employed to control post-harvest decay of citrus fruits. Penicillium rot and Diplodia or Phomopsis-rot of oranges is controlled by a 5 min dip in 6-8% alkaline suspension of borax. The effectiveness is improved by heating the solution

to 43.5°C. Applicability of borax treatment is limited by its low solubility and disposal of toxic water after fruit wash since fruits must be washed to remove borax residue. Subsequently, a widely used combination was 4% borax + 2 % boric acid at 43.5°C. Later, borax was replaced by sodium ortho-phenylphenate as a more effective chemical against Penicillium and stem-end rots of citrus fruits.

Chlorine has been used in water for washing the fruits and destroying superficially present fungal propagules. A 10 min dip in 2% bleaching powder solution is reported to keep the citrus fruits free from green mold decay for a long time. However, chlorine is not effective against established infections. It is generally recommended only for purifying the water used for washing fruits, containers, etc. in large establishments to reduce inoculum load.

Singh and Khanna (191) had reported that very low concentrations of copper sulphate are toxic to *Alternaria tenuis* causing black core rot of mandarins. They had also reported that zinc sulphate, ferrous sulphate, and boric acid also suppress spore germination. Kumar and Grover (116) had recommended fruit dip in Bordeaux mixture against Alternaria rot of oranges. The disadvantage with copper based fungicides is that they leave stains on the fruit skin.

Among the heterocyclic nitrogen compounds or dicarboximides, Captan considerably delays the blue and green mold rots of citrus fruits if used at 0.2% (10 min dip). Fruit dip for oranges in 5 ppm Phaltan (folpet) is effective against *A. citri* (116). The wettable powders are generally not recommended for post-harvest fruit treatment because they leave visible residue on the fruits.

Biphenyl (diphenyl) is a major fungicide for treatments of citrus fruits in many ways. The crystalline compound sublimes in the packed containers, fumigating the fruits during the entire period of transport and storage. It has a strong fungistatic effect against *P. digitatum, P. italicum, Diplodia natalensis, Phomopsis citri,* etc. The growth and sporulation of *Penicillium* on fruit surface is inhibited and the spread of decay to adjacent fruits is prevented in the containers. However, biphenyl does not affect bacteria, yeasts, Phycomycetous fungi and the resistant strains of *Penicillium* and *Diplodia*. It also does not control decay caused by *Geotrichum candidum* (sour rot), *A. citri* (black rot) and *C. gloeosporioides* (anthracnose rot). The most common use of biphenyl has been in the preparation of fungicide-impregnated paper sheets for packing, one sheet at the floor of packing case and another at the top.

Isolates of *Penicillium* sp. and *Diplodia natalensis* may develop stable resistance to biphenyl by constant exposure to this compound and failure of the treatment has been reported (98, 198). Biphenyl-resistant strains of

Penicillium may also develop by exposure to the chemically related orthophenylphenol and orthophenylphenol-resistance can develop by prolonged exposure to biphenyl. Pre-storage treatment of lemons with orthophenylphenate is now discontinued in USA.

Although biphenyl has been highly successful in controlling major fruit decays of citrus and is still used worldwide, there have been certain reservations about its use from the very beginning. One is the odour it leaves on treated fruits which the consumer does not like. This odour is temporary and dissipates in a few days. The other adverse effect is that it accelerates physiological decline of the fruit button (calyx + receptacle). This predisposes lemon fruits to attack of *A. citri*. In spite of these drawbacks use of biphenyl has continued throughout the world. The residue remaining on the fruit is safe from a toxicological viewpoint and the intensity of odour has no relationship with residue on the fruit (64).

Orthophenylphenols and sodium orthophenyl phenate came in to use for better volatility than biphenyl, reducing the unpleasant odour and for the same type of decay control as biphenyl. Citrus fruits are submerged or flooded with 0.5% alkaline (pH 11.5-11.8) solutions of sodium orthophenylphenate or the fungicide is incorporated in wax coating of fruits. In solution treatments the fruits are washed to prevent phytotoxicity.

To combat strains resistant to biphenyl and orthophenylphenol, the systemic fungicides of benzimidazole group such as thiabendazole (TBZ), benomyl (Benlate), carbendazim or MBC (Bavistin), thiophanates, and some of the sterol-biosynthesis inhibiting (SBI) triazole fungicides such as triforine were introduced for post-harvest fruit treatment. TBZ was first used in 1967 against *P. digitatum* and benomyl in 1969 against *P. italicum*. Thiabendazole (0.075-0.1%) was used as fruit dip or as fruit wax preparation against Penicillium decay of oranges (28). Residue of thiabendazole on the surface of citrus fruits sprayed with 0.1% suspension prevents sporulation of *P. digitatum* on decaying fruits (64). Generally, the same concentrations of benomyl or carbendazim are also used. Benomyl has been used as pre-harvest grove spray and found effective against Penicillium rot. It persists in orange fruits (26).

In 1980s, imazalil (Fungaflor), an imidazole SBI fungicide, was found effective against *P. digitatum* on citrus fruits as 15 sec dip in 1000 μg/L water. It prevents production of spores on infected fruit which could contaminate and rot the healthy fruits in packing cartons. The fungicide is systemic in fruits and penetrates the rind to a depth of 2 mm and prevents *P. digitatum* from invading fruits through injuries occurring after harvest (27, 29). The penetration is better while the fruit is still wet after treatment. Imazalil is also added to fruit waxes and applied to fruits as

non-recovery sprays with good results. However, it has been shown that imazalil controls green mold (*P. digitatum*) better when used in water solution than when used in waxes which keep a major portion of the fungicide bound and prevent its penetration (25, 27, 29). Smilanick, *et al.* (194, 196) have reported that warm water suspension of imazalil is more effective. Antisporulant activity of the fungicide at 500 μg/ml in water solution at 37.8°C is superior to 4200 μg/ml in wax. Fenpropimorph, a morpholine SBI fungicides, is also effective against citrus sour rot and green and blue molds.

With the use of systemic fungicides, resistance to the above named fungicides in the fruit rot pathogens has also been reported. Bus, *et al.* (30) conducted a study of isolates of *P. digitatum* and *P. italicum* from mandarin (*C. reticulata*), orange (*C. sinensis*), lemon (*C. lemon*) and grapefruit (*C. paradisi*) from different geographic regions and found 37% isolates resistant to TBZ (10 mg/L), 34% resistant to benomyl (10 mg/L) and 17% resistant to imazalil (0.2 mg/L). Ninety per cent of isolates resistant to TBZ were resistant to benomyl also and 13% of these were resistant to imazalil. Eckert, *et al.* (65) also have reported resistance to imazalil in *P. digitatum*. Strains of *P. italicum* resistant to benzimidazoles are capable of surviving with sensitive strains even in the absence fungicide selection pressure (222). Khilare and Gangawane (109) have used medicinal plant extracts against *P. digitatum* (green mold of sweet orange). While extracts of margosa alone provided significant control, combination of plant extracts with thiophanate also significantly improved the efficacy of the latter against thiophanate-resistant strains.

Sodium carbonate (soda ash), though less toxic than borax to conidia of *P. digitatum* and *P. italicum*, is also used at 3-5% concentration for fruit treatment. It leaves sufficient alkaline residues in potential infection sites of the fruit surface to prevent establishment of the pathogens. Soda ash (sodium carbonate) treatments are equal to or superior to imazalil treatments. Soda ash reduces the green mold rot by more than 90% when applied to lemon 48 hours after inoculation (195). The most effective control is obtained by 1-2 min dip of fruits in hot (40.6° or 43.3°C) solution of 4-6% sodium carbonate even when the treatment is given 24 hours after inoculation of the fruit with *P. digitatum* (191). The use of soda ash for post-harvest fruit treatment against fungal decay is economical and non-hazardous. This treatment at high temperature (48°C) may pre-dispose lemon fruits to decay without visible symptoms.

Use of plant growth regulators in treatments aimed at host-pathogen interaction to control post-harvest decay of citrus and other fruits has been reported. Spores of *A. citri* are present under the buttons (calyx + receptacle at the stem-end) of most lemon fruits at the time of harvest.

Senescence of this part of the fruit precedes the onset of Alternaria stem-end rot during storage (*cf.* 64). It has been demonstrated that 2,4-D and 2,4,5-T at concentrations of 100-1000 ppm in a wax emulsion applied to lemons after harvest reduce button deterioration, rate of correlation, and water loss by the fruit during storage. Since these chemicals are only weakly fungistatic for *A. citri*, the control of Alternaria rot is attributed to physiologic action on the fruit tissue. The albido of the rind of lemons possesses considerable resistance to *A. citri* but during ripening this resistance is decreased. Pre-storage treatment of fruits with 2,4-D delays this loss of resistance by the fruit rind. Half min dip in 25 ppm solution of 2,4-D with 2% wax solution is reported to give protection to citrus fruits against stem-end rot (*Diplodia, Botryodiplodia, Phomopsis, Alternaria*) and also blue, green and black molds. Combination of 2,4-D and Bavistin used as 1 min dip is an effective safeguard against storage decay of sweet orange. Gibberellic acid also delays maturity and senescence of rind of oranges on trees sprays with this chemical before harvest. In China, the antitranspirant compound called 'gao-zhi-mo' was used as dip for effective control (upto 82%) of storage decay of sweet oranges (97).

5. Biological Control of Fruit Rots

Biological controls have been developed that are effective against major post-harvest pathogens of citrus (and other fruits and vegetables). Fungal and bacterial antagonists used as alternatives to fungicides for control of fruit rots of citrus, apples, peaches, pears, and cherries include *Bacillus subtilis, Enterobacter cloacae, Pseudomonas cepacia, Pseudomonas syringae, Trichoderma* spp., *Acremonium brevae* and several species of yeasts (237). Their commercial application is limited by lack of proper formulations, except for *Bacillus subtilis*. *B. subtilis* as a biocontrol agent (fruit dip in cell suspension) can inhibit 10 citrus fruit pathogens by antibiotic production (192). These include *Lasiodiplodia theobromae*. *Trichoderma viride* is effective against Penicillium rots of citrus. Application of *Pseudomonas cepacia* (as fruit dip) to lemons after harvest gives 80% control of green mold without any visible injury to the fruit. *Pseudomonas fluorescens*, although ineffective against *P. digitatum in vitro*, reduces decay of lemons by 70% (193). The yeast, *Debaromyces hansenii* (now renamed *Candida guilliermondii*), when applied before infection can protect citrus fruits against green and blue molds and sour rot for 21 days at 11°C. The yeast is more effective against green mold than the other two rots (37, 141). The efficacy of the treatment is enhanced if the yeast suspension is prepared in 2% calcium chloride solution. The yeasts control fruit rot pathogen by competition for nutrition and, to some extent, by pathogen cell wall degradation (135). Glucanase activity as the main mechanism is reported.

■ CITRUS CANKER

Citrus canker or Asiatic citrus canker (also known as true canker or A-form canker) is a widespread disease in all the citrus growing areas of the world. It is reported to have originated from China but in the herbaria of the Royal Botanic Gardens Kew, England canker lesions have been detected in *Citrus medica* specimen collected from India as early as 1827-1831 and in *Citrus aurantifolia* specimen collected from Indonesia in 1842-1844 (*cf.* 70, 83). Thus, the origin of the disease is supposed to be in the tropical areas of Asia, such as South China, Indonesia and India where *Citrus* species are presumed to have originated. The pathogen was distributed through planting material and spread to Europe and to USA (in 1910) and to other citrus growing areas of the world. Citrus canker is found in Africa, Asia, Australia, Oceania and North and South America. It is a major disease of citrus in India, China, Japan and Java. It is economically important because fruit lesions downgrade the appearance of fruits and when severe, cause premature fruit drop. Heavy foliage infection causes severe defoliation, leaving only bare twigs. Severe infections of newly planted stock may cause delay in growth and can also be fatal.

Although the disease was once reported to have been eradicated from Australia, New Zealand, South Africa and the United States, its reappearance was reported during the 1980s in some parts of Australia, Mexico and Florida. In the state of Florida in the USA where it was first recognized as a new disease in 1913, it became so severe that mass eradication of diseased trees and nursery stock, often the entire orchard, had to be undertaken to eradicate the pathogen from the state in about 10 yrs (100). It was claimed that citrus canker had been completely eliminated from USA by 1949. However, it has reappeared in Florida since 1984 and the same eradication measures had to be started. The form of citrus canker which reappeared in Mexico and Florida seemed to be different from that identified in Asia (the A-form canker).

Symptoms

The disease occurs on leaves, twigs, thorns, older branches and fruits as necrotic brown spots with rough surface. Fawcett (68) had mentioned canker incidence on exposed roots. In the tropical climate of south India, Reddy and Naidu (168) reported canker lesions on roots of 5 yrs old Kagzi lime (*C. aurantifolia*) seedling plants upto a depth of 70 cm and in a 20 cm radius. This root infection caused decline of the plants.

Leaf lesions first appear as small, round, watery, translucent spots. They are raised and become yellowish brown. They first develop on the lower surface of the leaf and then on both the surfaces. As the disease

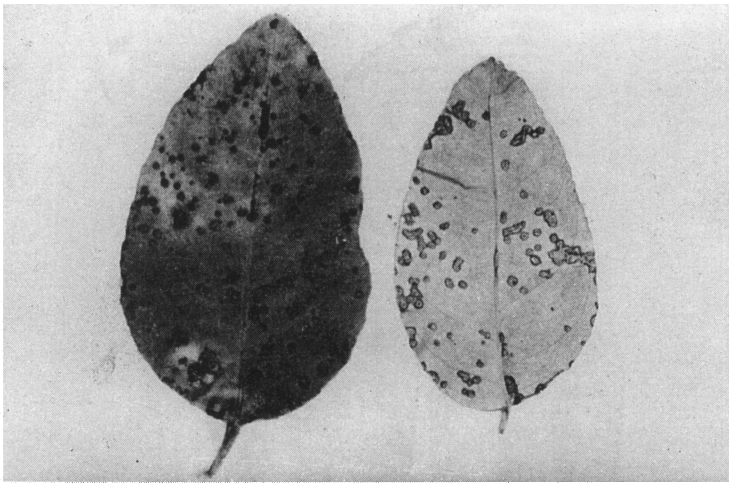

Fig. 3. Citrus canker on leaves.

advances the surface of the spots becomes white or greyish and finally ruptures in the centre giving a rough, corky, or canker-like appearance. The spots increase in size (1 mm to 1 cm in dia) and may coalesce to form elongated lesions on fruits and twigs. The rough lesions are surrounded by a yellowish-brown to green raised margin and watery yellow halo. Spots occurring on petioles and midrib cause premature defoliation. On larger branches the cankers are irregular, more rough and more prominent. Cankers on fruits are similar to those on leaves except that the yellow halo is not visible and a crater-like depression in the centre is more prominent. The injury to fruits is only skin deep and no visible effect on pulp or juice is noticed. Cankers on twigs cause them to break. The leaves during their early stage of formation and fruits of about 2-4 cm diameter are most susceptible to infection by the bacterium (88). As the fruits increase in size they become resistant but water soaking and lesion formation continues to occur as long as the fruit is expanding.

Histopathology

Citrus canker lesions are characterized by over-development of parenchymatous tissues, each consisting of a large number of hypertrophic cells and a limited number of hyperplastic cells. In the early stages of invasion by the bacterium, the spongy cells near the site of infection show increased size as well as increase in the amount of cytoplasm, followed by rapid enlargement. The hypertrophic cells occupy the intercellular spaces. As the cells further increase in size the callus tissue expands, lifting the epidermis above the leaf surface and finally causing

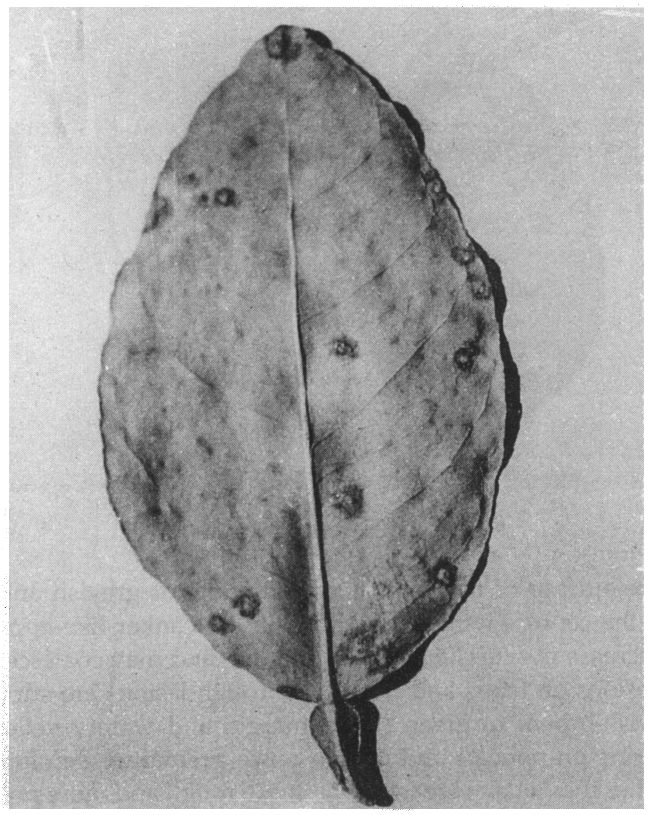

Fig. 4. Citrus canker. Close up of pustules.

disrution of the epidermis, exposing the internal callus tissue. Hyperplasia usually occurs in a few cells adjacent to the healthy tissue. The hyperplastic cells develop into hypertrophic cells without continuous cell division (83).

The Causal Organism

The bacterium causing citrus canker was known as *Xanthomonas campestris* pv. *citri* (Hasse) Dye [*X. citri* (Hasse) Dowson]. Gabriel *et al.* (76) had proposed reinstatement of the name *Xanthomonas citri*. However, Young *et al.* (239) had cautioned against the proposal. In the reclassification of *Xanthomonas*, Vauterin *et al.* (227) finally gave the name *Xanthomonas axonopodis* pv. *citri* (Hasse) Vauterin *et al.* A bacterial leaf spot of citrus is caused by *X. campestris* pv. *citrumelo* which was earlier considered a strain of *X. citri*.

The cells of the bacterium are rod-shaped and measure 0.5-0.75 × 1.5-2.0 μm. It forms chains and capsules but no spores and the cells are

motile by a single polar flagellum (monotrichous). It is Gram-negative and aerobic. Colonies on beef agar are circular, straw yellow to amber yellow, slightly raised and glistening. The yellow pigment is the characteristic xanthomonadin. Oxidase test is weak or negative. Litmus milk turns blue and milk is peptonized without coagulation. Asparagine is not used as a sole source of nitrogen and carbon. The cells are positive for hydrolysis of starch, aesculin, casein, liquefaction of gelatin and pectate gel, and production of tyrosinase and reducing substances from sucrose and hydrogen sulphide. The bacterium is negative for arginine dihydrolase, nitrate reduction, production of 2-ketoglucanate, acetoin, urease and amino acid dehydrolases and for methyl red test. Growth requires methionine or cysteine and is inhibited by serine. Growth is inhibited by 0.02% trephenyl tetrazolium chloride and by 4% sodium chloride but not by 3% sodium chloride. Xylose, glucose, fructose, sucrose, galactose, mannose, maltose, lactose, trehalose, glycerol, dextrin, starch, malonate citrate, succinate and malate are utilized as the sole source of carbon. L-arabinose, rhamnose, raffinose, malicin, sorbitol, inositol, dulcitol, inulin, gluconate, oxalate, acetate and tartrate are not utilized. Optimal growth temperature is 28°C, minimal 6°-7°C, and maximal 36°-38°C. The doubling time is 79 min.

Fig. 5. Citrus canker on fruits.
Courtesy: Dr. H.B. Singh

Pathotypes within *X. c.* pv. *citri* (*X. axonopodis* pv. *citri*) have been identified by host range, geographic origin, bacteriophage sensitivities, plasmid content, and serology (*cf.* 83, 99). Pathotype A (the A-form canker) has the widest host range and a global distribution. Pathotypes B, C, and D are restricted to lemon (*Citrus lemon*) and lime (*Citrus aurantifolia*) in South America and Mexico. Three phages of the bacterium have been isolated and characterized. The phage susceptibility can be used for rapid identification of the citrus canker pathogen. Strains of citrus canker bacterium carry indigenous plasmids (42). The function of these plasmids is not yet clear. However, plasmids differ in size among different strains (A, B, C). Differentiation based on plasmids (99) is consistent with that observed on the basis of serology and of phage susceptibility.

Disease cycle

Studies have shown that the bacterium of citrus canker has a short life in soil or in fallen leaves. The short longevity in natural soil is attributed to microbial interactions, especially the predatory effect of protozoa (83). This is generally true when the temperature is warm enough to allow the soil microorganisms to compete with the bacterium. Graham *et al.* (90) have reported populations of the bacterium in soil in citrus nurseries in Maryland (USA) and in Argentina, both temperate climate regions. When the source of inoculum is removed (removal of affected leaves and twigs) the survival in soil is considerably reduced. In presence of active host tissue for support such as roots it can survive deep in soil (172). In tissues of fallen diseased leaves and twigs also the bacterium dies quickly. The survival is not for more than 3 weeks if the lesion-bearing leaves and twigs are wetted on the soil surface or are buried at a depth of 3-6 cm. If the plant debris is maintained under dry conditions the survival is increased to 2-3 months.

The extracellular polysachharides (EPS) which form a matrix in which bacterial cells are embedded play an important role in survival of the bacterium. If the EPS dries and is not disturbed the survival of the bacterial cell can be considerably prolonged because EPS forms a protective coat that prevents desiccation of the cells. When the EPS matrix is diluted with water to the level of lower than a million cells/ml the cells are rapidly killed. This lethal dilution effect is found in most xanthomonads and some other bacteria. It also implies that during dispersal by raindrop splashes the bacterial cells must carry with them sufficient EPS to remain viable (83).

Although the bacterium has been detected on certain weeds, this source does not support prolonged suvival. Epiphytic populations on citrus leaves away from lesions also quickly decline. However, since the host is a

perennial plant the cankers on it can support parasitic survival of the pathogen indefinitely once the plant gets infected (49, 82, 83). In temperate climate countries where the citrus trees undergo dormancy during winter, survival in holdover cankers on the trees is most important. In the subtropical and tropical countries such as in India attacked twigs bearing old lesions on the standing trees are the main source of perennation of the pathogen.

According to Timmer *et al.* (208) when water is placed on lesions of detached leaves 10 to 100 thousand bacteria/ml from each lesion are released immediately. Exudation continues at a high level for 24 hr. Cumulative release is 100 thousand to 1000 thousand cells per lesion. Fewer bacteria are released and exuded more slowly from old lesions than from young lesions. The concentration of bacterial cells is largely dependent on the age of the lesions. In fresh lesions, bacterial density often reaches 10-100 million/drop (83). Individual canker lesions ranging 3-9 mm in diameter contain 1-10 million bacteria per lesion and lesions formed in spring flush continue to contain this level through summer and the rains (212). In winter the numbers decline.

In dispersal of the bacteria by rain, either the water running on the host surface or splashes of raindrops disperse the bacterium (49). In citrus nurseries with citrus canker, dissemination of the bacterium is primarily by splash dispersal. Rains driven by wind velocity in excess of 8 m/sec aid in dispersal (88). The bacterial numbers per ml of canker washings and their dispersal by rain-driven water splashes are, to some extent, effective as source of inoculum only if sufficient quantity of EPS accompanies the bacterial cells otherwise they may be desiccated before reaching the site for infection.

The role of strong winds has great importance in the epidemic of citrus canker. Strong winds cause many injuries to leaves and twigs. The nature of wounds varies from easily visible large wounds to small, invisible ones, such as small scratches or removal of the cuticle edges extending over the stomata. However, a single cell of the bacterium is enough to cause infection and the size of wounds does not matter. Storms help in long distance dispersal of raindrop-borne bacterial cells. In addition to driving rains, insects such as the citrus leaf minors (*Phyllocnistis citrella* and *Thosconyrsa citri*) also help in dissemination of the bacterium. Venkataswarlu and Ramapandu (228) have reported that the percentage of leaves affected by canker in presence of injury by leaf minors was 26 to 48 while in absence of the leaf minor injury it was 3 to 10. Man himself is the chief agent of dissemination and introduction of the pathogen into new localities through the transfer of infected nursery stock.

The result of numerous cells reaching various sites on host surface is the development of numerous secondary foci which later coalesce to

form larger patches. The bacterium enters the host through natural openings (stomata) and through wounds such as those caused by insects, movements of thorns, etc. Susceptibility of leaves to infection through wounds is maximum when the ratio of leaf length at a given time to the length of the fully expanded leaf is 0.8 : 0.9 or more (*cf.* 83). Wounds sustained early in spring or late autumn take longer to heal and, therefore, expose the injured tissue to infection for a longer period of time. The greater the number and size of stomata per unit area, the greater the susceptibility of the organ. But the stomatal invasion by the bacterium is governed by developmental stage of the organ. In young organs such as leaves, stems, and fruits, the front cavity of the stomata has a wide opening because the thin cuticular layer of the epidermis is not enough to elongate the edges. As organs approach maturity and the tissues become harder, the cuticular layer of the epidermis becomes thicker so that the edges develop over the stoma, leaving a narrow opening between them. The slit is so narrow that surface tension prevents entry of rainwater carrying the pathogen into the opening of the mature stoma. Thus, availability of young stoma determines the susceptibility of leaves, stems and fruits. In very young leaves, just after emergence, the stoma are immature with no opening and, therefore, only slight infection occurs.

After entry, the bacteria multiply rapidly in the intercellular spaces, dissolve the middle lamella, and establish in the cortical region. The bacterial cells which enter the intercellular spaces adhere to the host cell walls through and interaction between EPS and citrus agglutinins (204, 205). The citrus agglutinins contain 96% proteins and 4% carbohydrates. They are active with EPS of various xanthomonads at pH lower than 6.0. The EPS induces localized water congestion enhancing the growth of the bacterium in the intercellular spaces.

In association with EPS, the bacteria show ethylene biosynthesis for several hours after inoculation (84, 85). Continuance of ethylene production is followed by leakage of electrolytes (86) and amino acids from the cells indicating damage to cell membrane. A large amount of ethylene is also produced after canker symptoms develop. At this stage ethylene production originates in the hypertrophic host cells within the canker lesions as well as the cells in the peripheral zones, which appear to be under the influence of auxin and sometimes form yellow halo. The high level of ethylene produced at this stage induces the formation of abscission layer at the base of the leaf petiole, resulting in defoliation. Presence of antimicrobial compounds (phytoalexins) in citrus cells has been reported. However, these compounds are present inside the cells and are not leaked into the intercellular spaces to act against the bacterium. Citrusnin-A is one such compound (231).

The disease is favoured by mild temperatures and wet weather. Temperatures between 20° and 30° C with good evenly distributed rains are most suitable. Presence of free moisture on the host surface for at least 20 min is essential for successful infection. The size, density, and age of stomata determine susceptibility and resistance of a citrus cultivar. Under unbalanced conditions of excess nitrogen, citrus trees produce more shoots allowing an increased number of large and tender leaves which bear larger lesions and cankers.

Management of the disease

The only effective method of control of citrus canker is complete destruction of the affected trees by burning (56). Though drastic and costly, this method has proved its efficacy in USA, Australia, South Africa, New Zealand, and Brazil. The new eradication programme followed in Florida, after reappearance of canker involves 1) burning of plants in nursery where an infected plant is found, 2) destroying all trees with canker symptoms within orchards and defoliation of surrounding trees, and 3) using fruits from diseased or exposed trees for only processing (89). Similar rigid eradication programmes had been implemented in Australia where the first and subsequent outbreaks of the disease had been eliminated by this method. In spite of total destruction of infected and suspected trees and apparent elimination of the citrus canker from the country, the reappearance or reintroduction of the pathogen in the same country has pointed out the importance and possible failure of quarantine regulations. Two documented examples are of Florida (USA) and Australia. The introduction of Asiatic citrus canker in to Florida during the 1910s was traced to infected trifoliate orange seedlings imported from Japan for use as rootstock (*cf.* 89). The reappearance in around 1984 in Florida was again traced to entry of canker affected citrus. Detection of infected material had been made during 1973-1983 at the ports of entry. In Australia also, the first outbreak of canker in 1912 was attributed to citrus trees imported from Japan and China along with fruits. Mass eradication of trees and rigid quarantine had eliminated the disease but subsequent outbreaks in 1981, 1984, and 1991 are suspected to have originated from illegal importation of citrus into isolated home gardens in one part of the country and subsequent spread to other parts (23). Both these countries had followed the method of mass destruction of trees and imposition of rigid quarantine regulations not only at the international level but also within the country. In India and in other areas where the disease is well established in most orchards, eradication of trees as a control measure is not feasible.

Since wind-driven rains and water soaking of tissues are essential for dissemination and ingress of bacteria and for epidemic development of

citrus canker, wind breaks are essential. In absence of this precaution chemical and other methods of disease management remain inadequate (89). Other recommendations made to check the disease are: (i) use of disease free nursery stock for planting in new orchards, (ii) spraying the plants before planting in new orchards with a copper fungicide, and (iii) in old orchards pruning of the affected twigs and spraying with copper fungicides at periodical intervals, especially during the rainy season. The fallen canker affected twigs and leaves should be collected and burnt. Since inoculum present on fallen leaves is reduced when the leaves are buried deep in soil, periodical ploughing of the orchard floor is helpful. The plant vigour should always be maintained by suitable fertilizers and irrigation. Proper care should be taken to check the attack of leaf miners.

Rangaswamy *et al.* (169) had demonstrated that in orchards the disease could be controlled by antibiotic sprays. Streptomycin sulphate or crude agricultural preparations of streptomycin at 100 to 1000 ppm concentration sprayed at 15-day interval effectively checked the disease on 48-yr old lime trees while Bordeaux mixture was ineffective. Phytomycin (2500 ppm) was also effective. Four sprays of streptomycin-100 at 500 ppm are most effective in controlling the disease (114). In general, use of Bordeaux mixture (4 g copper sulphate with 4 g lime in 1 lit water) and Agrimycin or streptomycin is recommended in most countries. In China spraying citrus trees with copper ammonium during the summer and autumn is reported to reduce canker incidence by 86% and 90%, respectively. This control method is environment friendly, easy and cheap. Graham and Gottwald (89) have recommended chemical dip of fruits for shipment to prevent spread of the disease through infected or contaminated fruits. The treatments include 2-min dip in chlorine (200 μg/ml at pH 7) or 1-min dip in 2.0% sodium-*o*-phenylphenate.

Although chemical control has been claimed effective it is not very successful on all occasions. During rainy conditions, some bacterial cells may achieve direct access to the front cavity of stomata or wounds without being exposed to the chemical left on the leaf surface. This direct ingress of even very low number of bacterial cells may make the chemical sprays less effective (83). An effective bactericide against the citrus canker bacterium should not only be effective on the host surface but also reach into the substomatal cavity.

Studies on biological control of citrus canker are in a preliminary stage. A strain of *Pseudomonas syringae* is reported to show anagonism to the citrus canker bacterium and also prevents enlargement of canker lesions as well as subsequent defoliation of infected leaves (161). The antagonist probably stimulates phytoalexin (citrusnins) synthesis in the tissues. Strains of *Pseudomonas fluorescens* also are strong antagonists of *X. citri.* Kalita *et al.* (102) isolated *Bacillus subtilis* and *Aspergillus terreus*

from phylloplane of citrus and reported that a strain of *B. subtilis* when sprayed on leaves in high concentration (100 million cfu) reduced canker incidence by 61.9%. A strain of *A. terreus* also reduced disease incidence by 47.5%. *Erwinia herbicola*, a common phylloplane microflora, grows more rapidly than the canker bacterium both *in vitro* and *in vivo* and eventually causes quick decline of the pathogen population (87). However, this bacterium grows only in the area where hypertrophic cells are established, but never in the front boundaries at which the pathogen attacks healthy tissues inducing development of hypertrophic cells. In the state of Andhra Pradesh in India, S. Vaheeduddin had reported in 1959 that spray of neem (margosa) seed cake at the rate of 80 kg/acre is highly effective against citrus canker as well as leaf miner. About 25 kg of the cake is soaked in 100 lit of water and allowed to decompose for a week. It is then sprayed without filtration. Some of the cake falls on the ground and becomes manure. Several sprays are required to produce good results. In experiments, two sprays at 3-week intervals during August-September reduced the disease from 5.8% in unsprayed plots to 2.5% in sprayed plots. Bordeaux mixture was not so effective. This control of canker was probably through enhanced microbial activity on the leaf surface which acted as a biocontrol agent.

Different species of *Citrus* show different degrees of susceptibility to the disease. These differences result from pathogen strain-host species interaction, ability of the host tissue to release phytoalexins in the intercellular spaces, behaviour of the stomata, level of density and size of stomata, presence or absence of thorns that cause injury to leaves and young twigs, etc. Reddy (171) had screened 144 varieties of *Citrus* spp. and related genera and has recognized 13 as immune to the disease.

■ CITRUS GREENING DISEASE

Citrus greening disease is common in Tanzania, South Africa, the Arabian Peninsula, India, Philippines, and many other countries (17, 110, 130, 142, 152, 153). In India, the disease is particularly serious in the northern states (152) and is considered more dangerous than tristeza (41) because of its widespread occurrence. In most trees greening is found along with tristeza. There is a synergistic relationship between tristeza virus and the bacterium of greening and the two are jointly responsible for citrus die-back and quick decline (130).

Symptoms

Citrus greening is characterized by yellows type of symptoms which are highly variable. Leaf chlorosis is the main symptom. It resembles the

symptoms of zinc deficiency. Since chlorosis can be caused by nutritional disorders many scientists have claimed control of greening with micronutrient sprays which only temporarily mask the symptoms of greening. In the yellow tissue of the leaf lamina scattered green islands are seen. The leaf veins are also yellow. A characteristic feature of greening is that the yellow areas are surrounded on one side by the midrib and on the other side by lateral veins. The yellowing expands towards the margins. The size of leaves is also reduced. The leaves are thicker than normal and usually remain erect. The internodes of branches are shortened giving a bushy appearance to the branch. Such branches produce an excess of buds and later show die-back. The diseased trees look stunted, flower earlier than the healthy trees and produce smaller fruits. There is a considerable reduction in the number of roots.

The Causal Organism

Citrus greening was considered a virus disease for a long time (140, 150-153). Later, it was considered to be caused by mycoplasma-like organism and some workers claimed its isolation and culture. However, in 1970, D. Lefleche and J.M. Bove' had reported that the disease was caused by a phloem-inhabiting fastidious bacterium with double-membrane cell wall, distinct from MLO cells (51). Later the bacterium was identified as *Liberobacter asiaticum* for Asian citrus greening and *L. africanum* for African citrus greening (Jogousix, Bove' and Garnier. *Mol. Cell Probes* 10: 43. 1996).

The cells of phloem restricted fastidious bacteria are Gram-negative, non-motile, non-pleomorphic, rigid rods measuring 1.0-2.0 × 0.2-0.5 (1.3 × 0.3) μm. These cells are present in mature sieve elements, irregularly distributed among vascular bundles. They are sensitive to different antibiotics including penicillin and tetracyclines. Due to the presence of the cell wall they are more sensitive to penicillin, which interferes with cell-wall synthesis, than to tetracycline.

The citrus greening bacterium is transmitted through vegetative propagation and by two species of citrus psylla : *Diaphorina citri* and *Trioza erytreae* (17, 33). Electron microscopic studies have confirmed the role of *T. erytreae* in transmission of the organism (142). Prevalence of these vectors determines the regional prevalence of greening. After acquiring the bacteria, the vectors remain infective for their whole life. A single individual is enough to transmit the disease. After being acquired by the vector the bacteria have an incubation period of 8-10 days in the vector body before the latter becomes infective. The vector can acquire the bacterium in its larval stage also but cannot transmit it to healthy trees (130). It can do so only after becoming an adult. Transmission of the

bacterium from sweet orange trees to periwinkle through dodder has also been reported (77).

Management

An integrated management system involving tree sanitation, production of disease-free nursery stock and eradication of insect vectors (110) is essential for control of the greening disease and also virus and viroid diseases of citrus that are transmitted by planting stock and insects. All badly affected and uneconomical trees should be cut down and destroyed. New plants raised from indexed stock or from nucellar seedlings (234) should be planted. In areas where greening affected trees and the vectors are present such healthy trees also become infected in a few years through the vectors. Eradication of citrus psylla by regular sprays of 0.02% of such insecticides as diazinon, endrin, or parathion reduces the spread of the disease. Kapoor and Cheema (103) and Cheema *et al.* (39) have reported 100% recovery of trees by spraying a mixture of Bavistin (carbendazim) and Ledermycin (500 ppm each) six times at 10 days intervals. Ledermycin alone is not effective while Bavistin alone reduces the disease incidence.

■ CITRUS TRISTEZA VIRUS

Tristeza, meaning sadness, caused by citrus tristeza virus (CTV), has been one of the most destructive diseases of citrus worldwide. The earliest report of the disease is from South Africa, about 1910, when it was considered as a quick decline of citrus scions propagated on sour orange rootstock (235). It was subsequently reported in Java in 1928, Argentina about 1931, Brazil in 1937 and USA in 1939. Later, similar diseases were reported from New Zealand, Australia, West Africa, Sri Lanka and Hawai. Now CTV is known to occur in most of the citrus growing areas of the world. It occurs throughout India and in neighbouring countries. Presence of the disease in India was suspected as early as 1953. It is presumed that the disease was present in the country even much earlier. In India tristeza is commonly associated with the greening disease (151). Synergism between the two has been reported (14, 130, 152). It has been speculated that tristeza originated in the Orient and was distributed worldwide by the movement of citrus budwood and plants. South Africa seems to be the first country to have imported the disease from the Orient (46). According to Wallace (229) nearly half the citrus trees in the world had been destroyed by tristeza up to the year 1959. Comprehensive reviews of the disease and the causal agent are available (7-9, 78, 118).

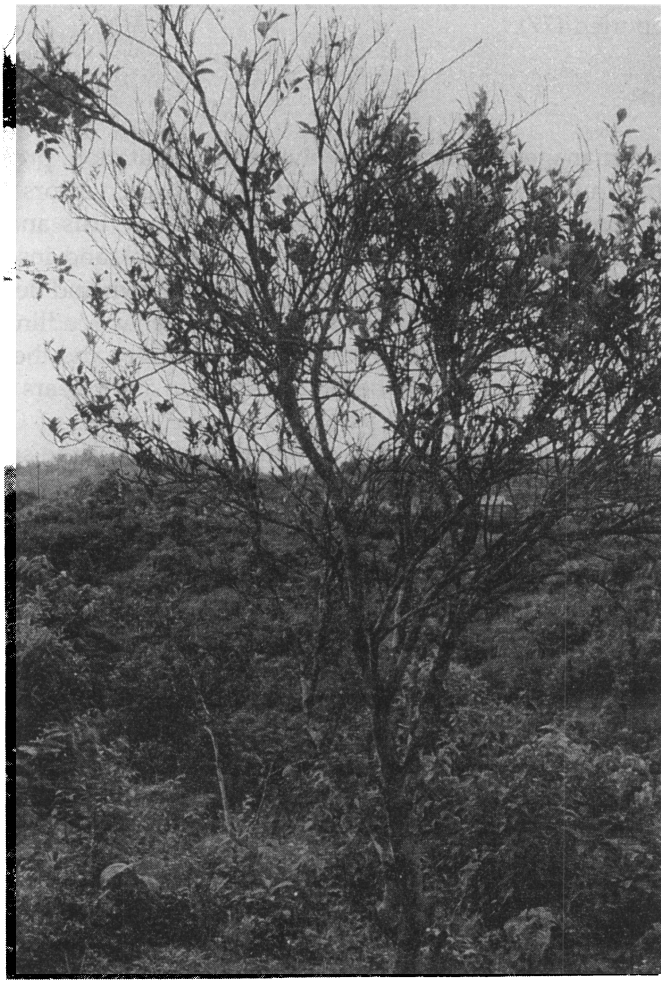

Fig. 6. Decline of citrus tree.
Courtesy: Dr. H.B. Singh

Symptoms

Tristeza is primarily a disease of sweet orange or other varieties grown on sour orange rootstock (235) and of grapefruit, lime and calamondin. The virus exists as many strains having different biological activities broadly grouped as mild, seedling yellows, decline on sour orange, stem pitting on grapefruit and stem pitting on sweet orange or no symptoms. Except for the mild or no symptom strains, the other categories of biological activity may occur alone or in any combination in a given CTV isolate.

The mild strains produce no noticeable effect on most commercial and commonly grown citrus varieties. In *Citrus aurantifolia* (Kagzi lime) mild strains cause only slight stem pitting, little or no vein clearing and flecking of leaf veins and veinlets. This host is an indicator plant for Indian CTV strains (32). In seedling yellows there is severe chlorosis and dwarfing of seedlings of sour orange (*C. aurantium*), lemon (*C. limon*) and grapefruit (*C. paradisi*). In decline of plants budded on sour orange rootstock, the plants are dwarfed, often chlorotic and show decline. The quick decline of sweet orange (*C. sinensis*), grapefruit or tangerine scions budded on sour orange rootstock can occur within 3-6 weeks. First the leaves turn yellow or golden colour and then they wilt and fall down, leaving only fruits hanging on the dead tree. An overgrowth just above the bud union can often be seen. If a piece of the bark is removed from the bud union, needle-like pegs originating from the bark (phloem) can be seen. Not all decline inducing strains of CTV cause a quick decline. Many cause a decline over a period of several years, causing the trees to stop growing and become less productive.

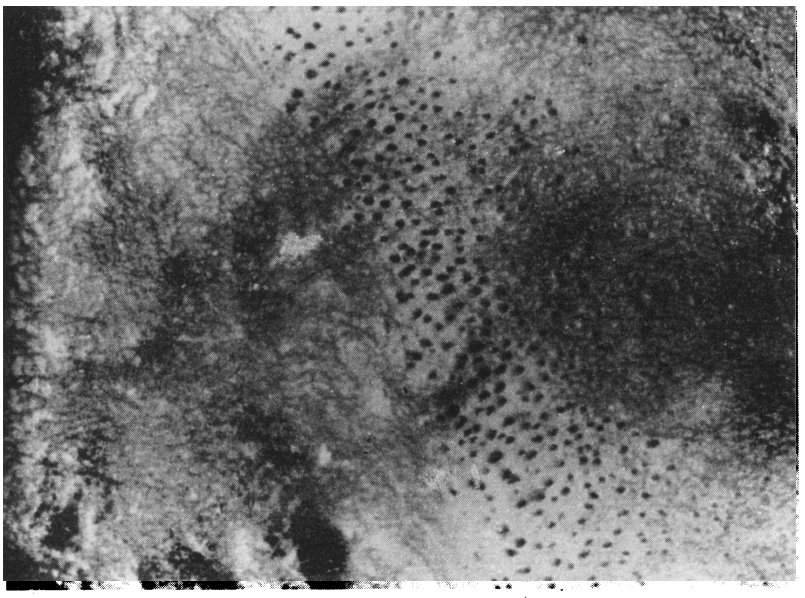

Fig. 7. Tristeza. Stem pitting.

Stem pitting is a common feature in grapefruit and sweet orange trees infected by CTV. When the bark is peeled off from the twigs pits can be seen on the wood. Plants also show stunting and chlorosis. In grapefruit, stunting can be very prominent producing large longitudinal ridges or

depressions running up and down the trunk giving the trunk a rope-like appearance. The trees develop a bushy top (mushroom-like appearance) with occasional branches growing away from rest of the tree. The stem pitting occurs on the scion regardless of rootstock. Fruit size and productivity is considerably reduced. In sweet orange trees same abnormalities occur. Twigs tend to be brittle and break easily. Strains causing stem pitting on sweet orange may cause stem pitting on rootstock also. A given CTV isolate causing stem pitting on sweet orange may or may not cause stem pitting on grapefruit and *vice versa*.

The CTV strains having the above mentioned biological activity are serologically related and provide cross protection. The mild strains have been used to provide cross protection against severe strains. In the recent past, however, new and destructive strains of CTV have appeared in parts of the world which are insensitive to cross protection and have resulted in decline epidemics.

The Causal Agent

The cause of the decline of citrus scion on sour orange rootstock was unknown for many years and was thought to be graft incompatibility between scion and stock (214) or a nutrition problem. Fawcett and Wallace (72), for the first time, recognized it as a virus disease transmitted by aphid vectors. CTV is a phloem-limited closterovirus (7-9). Quick decline symptoms are caused by necrosis of the phloem. The virus particles are flexuous filaments measuring 11 × 2000 nm in size. The CTV virions contain a single-stranded, plus-sense RNA with an estimated size of 5.4-6.5 million or about 20 kilobases (7). The only gene product which has been identified is the coat protein which accounts for 3% of the total coding capacity of CTV genome (119). The genome can code for atleast 19 protein products ranging from 6 to 401 kDa (104). Two coat proteins have been identified. The larger coat protein (CP1) has a molecular weight of 23000 to 28000 daltons and the smaller coat protein (CP2) has a molecular weight of about 21000 daltons (117, 119). Sedimentation coefficient of full particle of CTV is 105 to 131 S. The genetic system of CTV is complex. The infected plants contain the genomic RNA, at least 9 subgenomic RNAs and often multiple defective RNAs (104). In many cases the plants are doubly or multiply infected with genetically distinct CTV isolates or strains and there are indications of high recombination potential between different virus-specific RNAs. This complicates the control of CTV with cross-protection.

Closteroviruses are characterized by the occurrence of inclusion bodies that are confined to the phloem and associated tissues and appear as large aggregates in arrays that are often cross banded. In CTV such bodies

consisting of virus particles and related proteins occur in infected tissues (18). These inclusion bodies are found in phloem and occasionally in the ground meristem of newly forming stems. These may be seen with light microscopes after Azure A staining (20). The number of inclusion bodies is possibly related to strain severity and to virus titer (19). Within the plant the virus moves only through the phloem and fails to enter the xylem (35). Thus, the concentration of virus particles is highest in the phloem. In vectors the concentration of virus particles is low.

Transmission

CTV is transmitted by budding, grafting and by many aphid vectors (7). The aphid species transmit the virus in a semi-persistent manner (177). The virus is both stylet-borne and circulative. It is normally not sap transmissible. The first vector reported for CTV was the oriental citrus aphid, *Aphis citricidus*, now known as *Toxoptera citricida*. Later, other aphid species were reported as vectors. These include *Aphis gossypii*, *A. citricola*, *Toxoptera aurantii*, *A. craccivora*, *A. spiraecola*, and *Myzus persicae* but these are less efficient vectors. *T. citricida* (brown aphid) is the most efficient vector with 20% transmission by single aphid transfers. Geographic spread of tristeza with the spread of *T. citricida* in the Central American and contiguous countries has been reported (118). According to some recent studies efficiency of *A. gossypii* appears to have improved. There seems to be a lag period of about 30 years after introduction of CTV in a new area before A. *gossypii* becomes a relatively efficient vector. Perhaps, there is a helper factor which becomes widespread during the lag period and which enables the aphid to gain efficiency (*cf.* 118). Helper factors have been reported for other semi-persistently transmitted viruses. CTV has been transmitted from citrus to citrus by dodder (*Cuscuta subinclusa* and *C. reflexa*) also (173).

Costa and Grant (47) had reported that a 24-hour acquisition feeding was most effective for virus transmission by the vector although the virus can be acquired in a few seconds and transmitted in a few seconds of feeding. Aphids do not remain infective after feeding on healthy plants for 24 hour or longer. They lose the virus within 24 hour if not allowed to feed on healthy plants.

Management

The management of tristeza involves regulation, cultural management and biological control measures including mild strain cross protection, genetic engineering for virus resistance and breeding for virus resistance in commercially acceptable scions and rootstocks.

Management Measures for Citrus Tristeza Virus (114)

Prevalence	*Management measures available*
CTV absent	Quarantine; budwood certification and/or clean stock programmes
CTV present	
Low incidence	Eradication/suppression programmes; budwood certification; clean stock
High incidence	Tolerant rootstocks; varietal tolerance to stem pitting; mild strain cross protection; genetically engineered resistance; budwood certification

Regulatory measures

Movement of CTV-infected budwood or plants is the means by which tristeza has spread around the world. In areas where the disease is not present, the first line of defence against its entry is quarantine at international and national level. Any citrus planting material should be free from the virus before it is brought to a clean area. Clean stock and certification programmes for citrus serve a useful purpose in providing propagative material and nursery stock which are true to type and free from viruses and graft transmissible diseases (e.g. citrus greening). However, under special situations where importation of doubtful material is unavoidable the material should be made disease-free by heat therapy and/or shoot tip grafting followed by indexing for certification. Heat therapy can eliminate some pathogens, including CTV, but not the viroids (CEVd). Apical shoot tip culture or grafting can eliminate viroids as well as other pathogens. Methods combining pre-heat treatment followed by shoot tip culture/grafting in practiced in Japan (113) for elimination of known graft-transmissible pathogens of citrus.

Such clean-stock and certification programmes are very effective for viruses which do not have a vector in a clean area. They are less useful against disease which are vector transmitted such as tristeza. In India, tristeza is present in most orchards and the vector is also present. Thus, even if clean grafts are used for planting they soon become infected through the vectors. Therefore, eradicative and vector control steps may be warranted even if disease-free stock has been planted.

Cultural measures

These include use of CTV-tolerant rootstock, switching between resistant or tolerant cultivars such as between grapefruit and sweet orange and proper water management. CTV is inactivated in seedlings or budwood by exposure to 35°-43° C for 87-107 days (95).

True seeds of citrus do not carry the tristeza virus and these can yield virus-free plants. But such plants are not true to type and have no

commercial value. This problem was solved by obtaining seedlings from nucellus embryo which functions as a seed but has no virus (231). Such seedlings can be used either for direct planting or for raising resistant rootstock. Although, scientifically an encouraging programme, this approach also has limitations. First, the method takes 10-15 years to produce usable disease-free budwood for commercial propagation. Second, the existence of large contiguous orchards planted with a single variety promotes a rapid spread of viruses and virus-like agents. If the brown aphid is present in the area and if even a few infected plants are present in the locality, the plants raised from nucellar seedlings become infected in a couple of years. Providing chemical protection to the healthy plants also does not work because the insecticides do not instantly kill the vector which can transmit the virus in a very short time. In many countries, where the brown aphid is not common, rootstock raised by this method has been successfully used to reduce tristeza incidence. Waterlogging predisposes the plants to tristeza by killing or damaging roots.

Chemical protection

There is no direct chemical treatment against the virus. It has been suggested that insect control by chemicals over a large area may reduce spread of CTV by reducing aphid populations.

Biological Control

Disease resistance: Many citrus cultivars are tolerant of CTV infection meaning that CTV replicates in the host but no symptoms are expressed in infected plants (46). Some citrus relatives such as *Poncirus trifoliata* (trifoliate orange) are immune to CTV infection meaning that CTV does not replicate in the host (9). Trifoliate orange can be hybridized to citrus and many hybrid cultivars have been obtained which are immune to CTV replication. These cultivars are widely used as CTV-tolerant rootstocks (79). Somatic hybridization between protoplasts of sexually incompatible citrus relatives has also been successfully used to produce hybrids which can be used as tolerant rootstocks (96). However, such plants are not immune to CTV.

Mild strain cross protection: Cross protection against CTV usually deals with either decline on sour orange or protection against stem pitting. This has been a proven control strategy for citrus tristeza (48) and many other plant virus diseases (81). Mild strains are selected and inoculated on trees to provide protection against severe strains. In India, cross protection to CTV has been demonstrated in acid lime. The mild strains used must be mild in all citrus cultivars or susceptible hosts. Such viruses must be stable. There are reports that mild strain cross protection may

fail to prevent superinfection with certain severe strains of CTV (164). Within the orchard, spread of severe strains is much higher than the mild strains (165).

Possibility of genetically engineered protection

Genetic engineering has the potential for CTV management. There are several possible approaches (118). First, satellite RNAs of cucumber mosaic virus and tobacco ringspot virus have been cloned and these cRNAs are inserted into vectors which enable transformation of plant tissue. This approach has afforded some degree of virus resistance in the transgenic plants. Second, antisense RNAs of cucumber mosaic virus, tobacco mosaic virus and potato virus X when expressed in transgenic plants have shown a small degree of virus resistance. Third, the incorporation of the viral coat protein gene and expression of the virus coat protein in transgenic plants provides a high degree of resistance in the transgenic plants. The plants transformed with the viral coat protein genes result in plants showing the benefits of cross-protection without the virus being present hence the disadvantages encountered in mild strain cross-protection are eliminated. This approach also provides a more broad based protection against diverse strains of CTV.

■ XYLOPOROSIS OR CACHEXIA

This virus disease of citrus was first noticed in the Philippines in 1934. It is now a destructive disease in California and Florida (USA), Brazil and Argentina (140). On the basis of its occurrence at international level, it is considered next to tristeza. There are unconfirmed reports of its occurrence in India also. However, since the susceptible rootstock (sweet lime and Orlando tangelo) are not used in India, chances of its occurrence are low.

Even in susceptible hosts the symptoms appear late, usually when the trees are 3-4 years old. Initial visual, but non-specific, symptoms are sick and weak looking appearance of the trees and stunting of leaves and the tree. In comparison to healthy trees the diseased trees fruit early and rather heavily. The fruits have more sugars and acids. Later, the trees cease fruiting.

When bark at the bud union is removed, pits measuring 1-4 mm in diameter when circular or 1-10 mm long when triangular are seen on the wood of rootstock and scion. Matching these pits there is raised, peg-like tissue on the inner side of the bark. In susceptible hosts such as Orlando tangelo, these pits are full of a gum-like material but in others this gum is not common. In advanced stages of the disease, the bark splits at the bud

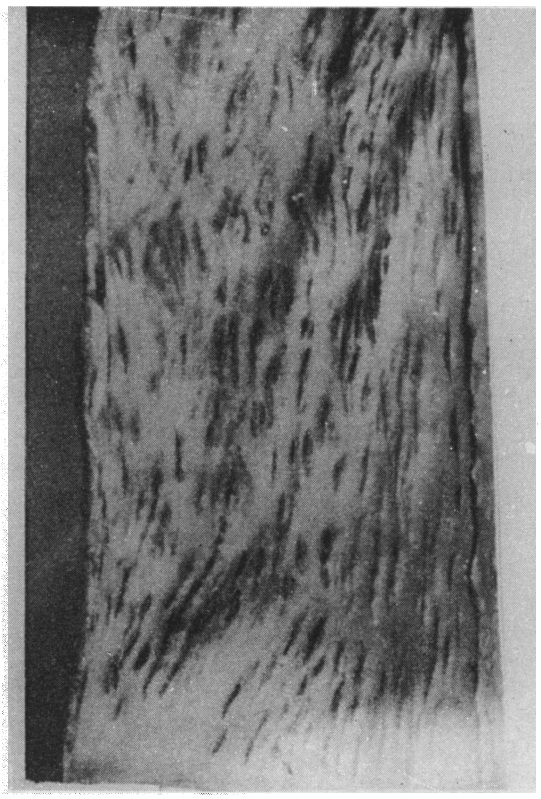

Fig. 8. Xyloporosis. Pits on wood under the bark.

union. The basal portion of the stem loses strength, easily bends and appears bending on one side. Due to blockage of food movement, the bud union appears swollen.

The virus is transmitted during budding. It can be managed if care is taken to select rootstock and scion only from sources that are totally virus-free. Precautions recommended against tristeza can also give satisfactory control of this virus.

■ PSOROSIS OR SCALY BARK

This virus disease is known under different names such as psorosis A, psorosis B, concave gum, blind pocket, crinkly leaf and infectious variegation. The disease is serious in USA and many other countries. According to McClean (140) in majority of citrus growing areas of the world the number of trees ceasing to fruit due to this disease is higher

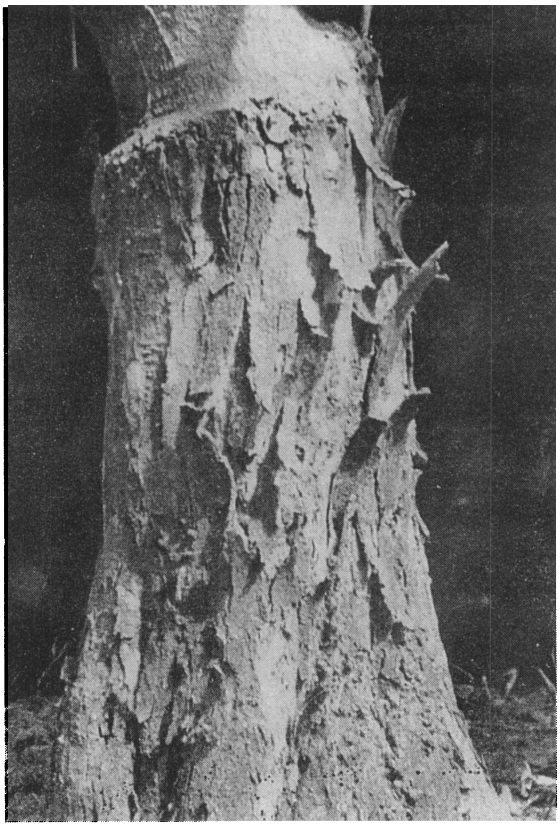

Fig. 9. Psorosis. Scales on the trunk.

than due to any other disease. The virus causing the disease has no relationship with the rootstock. Psorosis was reported in India in 1959 and is widespread in citrus orchards in the country (41).

The scaly bark is caused by strains of different viruses and symptoms are variable according to the strain involved. The common symptom is a bright yellow colour of the leaf veins. This is more conspicuous on new leaves. Leaf flecking also occurs all over the lamina. In the infection of psorosis A, characteristic scales develop on the bark of the trunk and thick branches. They may be absent in some cases. The bark scale is generally some shade of brown. The affected portion is spherical which first appears as pimples and progresses to girdle the entire stem or the branch. The outer 2 mm thick bark separates as a scale from the underlying living tissue. In advanced stages of the disease the entire bark and some part of the wood looks reddish brown. Sometimes gummosis also occurs. Decline of trees is very rapid when wood of the trunk or branches is also

affected. In psorosis B gummosis occurs before scaling of the bark. The scaly bark progresses upward. Bright yellowing of veins and translucent spots are present on old leaves also. Such spots may develop on fruits also. In blind pocket psorosis, deep parallel furrows with straight wall develop longitudinally on the tree trunk. Sometimes the bark develops scales and there may be gummosis. In concave gum psorosis these furrows are longer and broader and leaf veins are shining like oak leaves. Usually, the furrows are covered by the bark but it may sometimes crack and gum exudation may occur. In leaf crinkle psorosis leaves look crumpled.

The viruses causing different types of psorosis are transmitted through use of infected budwood (50). Some of them are seed-borne also (40). For control of this disease it has been suggested that scion material (budwood) should be taken only from 20-25 years old trees to ensure that they are healthy. Trees which are not fruiting satisfactorily should be cut down. Planting stock can be given thermal treatment by exposure to 35° - 43° C in a room for 87-107 days (95).

■ CITRUS EXOCORTIS VIROID

Exocortis is worldwide in distribution and affects trifoliate orange, citrange, Rangpur and other mandarins, sweet lime, some lemons and citrons. Orange, lemon, grapefruit and other citrus trees grafted on exocortis sensitive rootstocks show slight to great reduction in growth and yields are reduced by as much as 40%. Existence of exocortis in India was reported in 1968 (154, 163).

Infected susceptible plants show vertical splits in the bark and narrow, vertical, thin strips of partially loosened outer bark that gives the bark a cracked and scaly appearance. Since many of the exocortis-susceptible plants, such as trifoliate orange, are used primarily as rootstocks for other citrus trees, and because the scions make poor growth on such rootstocks, the enlarged, scaly rootstocks have given the disease the name "scaly butt". Infected exocortis-susceptible plants may also show yellow blotches on young infected stems, and some citrons show leaf and stem epinasty and cracking and darkening of leaf veins and petioles. All infected plants usually appear stunted to a smaller or greater extent and have lower yields (184).

The citrus exocortis viroid (CEVd) is similar to, but not identical with, potato spindle tuber viroid. It consists of 371 nucleotides arranged in a circular or linear form (54, 129). Host range of the viroid is largely restricted to the citrus family (Rutaceae) although solanaceous plants such as potato, tomato and petunia are also susceptible. There is no evidence of vector transmission. The viroid is readily transmitted from

diseased to healthy trees by budding knives, pruning shears, or other cutting tools, by hand, and possibly by scratching and gnawing of animals. It is also transmitted by dodder and by sap to *Gynura, Petunia,* and other herbaceous plants. On contaminated knife blades CEVd retains its infectivity for atleast 8 days and, when partially purified, CEVd remains infective at room temperature for several months. The thermal inactivation point of extracted sap is about 80°C for 10 min but partially purified CEVd remains ineffective even after boiling for 20 min. The viroid also survives brief heating of contaminated blades in the flame of a propane torch (blade temperature about 260°C) and flaming blades dipped in alcohol or on contaminated blades treated with almost all common chemical sterilants except sodium hypochlorite solution.

The viroid can be isolated from all plant parts including roots and fruits. Actively growing regions of the tree contain the highest concentration of the viroid. The viroid apparently enters the phloem elements and spreads in them throughout the plant. It is associated with the host nuclei and internal membranes of cells and results in aberrations of the plasma membrane. Although the viroid apparently lacks the ability to serve as a messenger molecule or as an amino acid acceptor, it brings about several metabolic changes in infected plants. These changes include an increase in oxygen uptake and respiration and also in sugars and certain enzymes. Marked changes also occur in several amino acids. Marigold (*Tagetes patula*) has been reported as an experimental host of the viroid (75).

Exocortis can be managed only by propagating healthy nursery trees from certified foundation stock and use of sanitary budding, nursery and field practices. Apical shoot tip culture or grafting eliminates the viroids and many other pathogens (113). Tools should be disinfected between cuts in different plants by dipping in a 10-20 % solution of household bleach (sodium hypochlorite).

■ CITRUS ROOT NEMATODE

The citrus root nematode was first noticed in 1912 on orange trees in California (USA). In 1913 the nematode was described by N.A. Cobb who created the genus *Tylenchulus* with the type species *Tylenchulus semipenetrans* for the nematode. The nematode is present throughout the world wherever citrus is grown. In the United States it is placed among the economically most important phytonematodes because of the reduced fruit yields and poor quality of fruits. In India, where the nematode was first reported in 1961, upto 80% orchards were found infested in certain

areas. The *Citrus* spp. found infected were *C. limon, C. sinensis, C. reticulata* and *C. aurantium.*

The nematode causes slow decline of citrus trees with die-back of twigs and debilitation of the trees. Normally, the trees are not killed but over the years they become nonproductive. Although, die back and decline could be caused by many other factors such as lack of moisture and nutrients, presence of hard pan in the subsoil, attack of bacteria (citrus greening) and virus (tristeza), the association of *Tylenchulus semipenetrans* as the major cause had been proved as early as 1923 (206).

Symptoms

Symptoms of slow decline are similar to those caused by poor nutrition. In early stages of root infection no above ground signs of the problem are visible. It is only after a few years that damage to roots show an adverse effect on the foliage. Affected trees show reduced terminal growth, chlorosis, shedding of terminal leaves, die-back of branches and considerable reduction in number and size of fruits. The fruit quality also deteriorates (15). Die-back symptoms first occur on the upper portion of the tree but later extend to the lower portion. Copper and zinc deficiency symptoms are more pronounced (15). Roots of such trees show brownish discolouration and unusual adherance of soil particles to the root surface. When roots are disturbed or gently pressed, the cortex readily separates from the axial portion. Ultimately, the roots decay resulting in reduced root volume. Up to 60% reduction in dry weight of root mass has been reported (224). The destruction of roots is not as much by the nematode invasion as by the invasion of secondary parasites such as *Fusarium solani* (225).

Morphology and Life Cycle

Tylenchulus semipenetrans is a sedentary semi-endoparasite of roots. Females are most commonly found on thick, stunted rootlets to which a layer of soil particles is clinging. These particles are held in place by a gelatinous mucus secreted by the female. The mucus and adhering soil particles protect the females and eggs hatched by them from their natural enemies. The young and egg-laying females can be seen in groups clinging to rootlets with their head and neck buried in root cortex.

Females are 0.35-0.40 mm long with a variable saccate body which is usually bent ventrally in vulvar region just anterior to the short, blunt tail. The excretory pore is unusually well developed and is located just anterior to the vulva. The single ovary has usually two flexures and reaches the esophageal region. The eggs average 33-67 microns in size. Usually only one egg is seen in the uterus at one time.

Larvae hatched from eggs under laboratory conditions in 1-14 days are of two types. One type is shorter and wider than the other (226). From the shorter and wider larvae, which do not feed, mature males develop within one week after three moults, one moult having occurred within the egg. Such males are about 26% of the total larval numbers. They measure 0.26-0.34 mm in different stages and are non-parasitic. The longer, more slender individuals fail to develop unless they feed on the host root. These become the females. After egg hatch, the second stage female larvae, require about 14 days to locate and feed on epidermal cells of host roots (223) until ready for moulting. During the period of searching for a feeding position 24-50 larvae congregate under fragments of cortical tissue, soil particles or organic matter. They are rarely seen on unprotected locations on the root. These second stage larvae measure 0.3-0.36 mm in length. This stage is most frequently found in soil samples and can live without host roots at a temperature of 15° C for more than a year but at 33° C only for four to five and half months.

The third and fourth stage female larvae are shorter with longer esophagus and a distinct vulval cleft. The length varies from 0.23 to 0.36 mm. Well developed spear measures 13.5 μm. The esophagus occupies almost half the body length. The vulva appears as a deep transverse slit. The excretory pore is very prominent and is located near the vulva. The tail is slightly convex-conoid dorsally, ending in a blunt terminus. The anus and rectum are not seen. The fourth stage female larvae and young females are seen about 21 days after inoculation. Within a week young females penetrate deeper in the cortex of the root, become sedentary and feed. The females excrete the gelatinous mucus in which eggs are deposited. Egg laying occurs 50 days after inoculation. The complete life-cycle from egg to egg requires 6-8 weeks at 25°C. Reproduction occurs without the help of males.

In the host root cortex, the feeding zone develops a nurse cell system consisting of uninucleate, not enlarged, discrete parenchyma cells (238). Synchytium is not formed. This type of nurse cells system is characteristic of this nematode. Feeding of the citrus nematode in cortical cells results in necroses. The injury does not extend to the stellar region of the root. Secondary invasion of the fungus pathogen *Fusarium solani* through injured tissue has been confirmed by many workers. Root decay in lemon by *F. solani* is increased when citrus nematode is also present (225). This could be an important factor in the lowering of tree vitality.

The spread of the nematode through soil is slow, the rate being approximately 15 cm per month when roots of adjacent trees are in contact. The nematode is, however, spread over long distances by movement of

nematode infested soil on equipments, animals, and by irrigation water and even to longer distances by transfer of infected citrus nursery plants. The population of the citrus nematode is closely related to the stage of decline of the trees. Usually, the population is maximum on healthy looking trees with early stage of root infection and minimum on trees in advanced stage of decline. Seasonal and regional variations at the time of peak population of the nematode on roots are reported (155, 167). These depend on host cultivar, temperature and rainfall. In the northern states of India populations are at the lowest during January and February while in Maharashtra (south) maximum populations are reported in these months.The nematodes are found in large numbers 30-50 cm from the main trunk of the tree in the top 30 cm of soil. The highest numbers are in top 20 cm but some can be found upto a depth of 100 cm or even more (187). The number of nematodes on the root surface is very large, 108 nematodes found on a 4 mm piece of root (15). Under a single tree their number can reach the figure of 700 million at 15-60 cm depth.

Infection of roots is severe in sandy loam soil. In soils with 50% clay, reproduction of the nematode is extremely slow. The highest rate of reproduction and the greatest reduction of plant growth occurs in soils with 10-15% clay. Oxygen supply is important for activity of the nematode. A decrease in infectivity is associated with a corresponding decrease in motility and body contents of second stage females which are aged and starved at 27°C in soil and water (222). Survival of infective larvae is better in soil than in water. Optimum temperature for infection, growth, and reproduction of the nematode is 25°-30° C. There is much less infection at 16° C and at 35° C. At 20° C infectivity is greater than at 30° C but reproduction at 20° C is delayed by 4 weeks (155). For reproduction optimum temperatures are 28°-31°C. Above 31°C there is no reproduction. Temperatures suitable for citrus root growth are favourable for the activity of the nematode (155).

Host nutrition also plays a role in the severity of attack of citrus root nematode. Citrus seedlings growing in soil containing calcium carbonate, sodium and potassium levels insufficient for satisfactory growth of the host show maximum attack of the nematode (224). Leaf copper is reduced in the infected trees. Low levels of phosphorus also retard seedling growth by the nematode. The population of the nematode increases under low phosphorus conditions while high nitrogen suppresses it. Root phosphorus in excess of 0.3% retards the numbers of the nematode. Naidu *et al.* (148) have reported that root exudates of acid lime (*C. aurantifolia*) infected with tristeza (CTV) caused 44.8 and 70.2% mortality of larvae by exposure for 12 and 24 hours, respectively.

All species of *Citrus* and allied genera are attacked by *T. semipenetrans*. Other hosts are *Andropogon rhizomatus, Diospyros lotus* (Ebenaceae, date

plum), *D. virginiana* (persimmon), *Makania batatifolia, Oleseuropea, Syringa vulgaris* (common lilac) and *Vitis vinifera* (grapes). The roots of *Citrus limon* (lemon), *C. aurantifolia* (lime), *C. medica* (citron) and *C. pennivesciculata* support very high populations of the nematode whereas *C. aurantium* supports the lowest population.

Management

Protection of rootstock is very important. Citrus nurseries should never be established on or near old citrus orchards. Nursery soil should be fumigated before planting for rootstock is done. Suspected rootstock should be denematized by hot water treatment at 45°C for 25 min. The seedling roots can also be treated by bare root dip method in nematicides such as zinophos (156, 157).

DBCP (Nemagon) had been extensively used for the control of citrus nematode. The nematicide was effective at a dosage of 15-45 lit a.i/ha and had reduced the population of the nematode by 98% with concurrent increase in fruit yield by 440-200% (34, 145). Fruit size in lemon increased by 11% with Nemagon treatment. This nematicide has been withdrawn from the market because of environmental pollution problem.

Dimethoate at 16 ml a.i. per tree and phorate (Thimet) at 15 g a.i. per tree followed by light irrigation are also reported to reduce nematode population by 82 and 90%, respectively, on sweet orange. Fensulfothion (Terracur P or Dasanit) at 24.7 kg a.i./ha, ethoprophos (Mocap) at 61.5 kg a.i./ha, and dichlofenthion at 27.8 lit a.i./ha give effective control of the nematode. Upto 90% reduction in nematode population and yield increase of up to 90% in the second year by application of ethoprophos at 40 kg a.i./ha are reported (145). Le Roux *et al.* (120) have recommended soil application of aldicarb (Temic) at 5 g a.i. per sq. metre of leaf canopy. Treatment was given at 3 months interval in 24 months. After 32-44 months significant reduction in root decay and increase in fruit yield was recorded.

Many reports suggest beneficial effect of organic amendment of the orchard soil. Steer manure, chicken manure, cotton waste, sugar beet pulp, lucerne hay and castor pomace (cake) are reported to reduce the population of citrus nematode (127, 128). Singh (187) obtained reduction in nematode population by applying castor cake at the rate of 10 kg/tree in trenches 30 cm wide and 30 cm deep on the periphery of the trees. Amendments with easily decomposable nitrogenous materials enhance microbial activity including microbivorus and fungivorus nematodes. The suppression of nematodes is correlated with high nitrate levels and

decrease in soil pH. Mankau (127) had isolated nematophagus fungi *Arthrobotrys, Dactylella,* and *Dactylaria* from amended soils. Cultivation of marigold in citrus orchards (in the drip area) is reported to suppress nematode populations.

The use of resistant or tolerant rootstocks against the citrus nematodes is the most practical method of management. All species of *Citrus* and related genera are not equally good hosts of the nematode and a certain degree of resistance does exist in some species and hybrids. Trifoliate orange (*Poncirus trifoliata*) and Troyer citrange (*P. trifoliata x C. sinensis*) are resistant to the citrus nematode and their use as rootstock is one way of managing this nematode.

■ SPREADING DECLINE OF CITRUS

This disease is reported only from the Florida state of USA although the nematode species associated with it is universally present on other hosts including banana. The disease is a famous example of devastation caused by a phytonematode. It was first noticed in between 1926 and 1928 in a citrus orchard of Florida. The disease expanded to more areas by 1935-36. Final proof of *Radopholus similis* being the cause was given by Suit and Du Charme (201). The loss in fruit yield to the extent of 50-80% in grapefruit (*C. paradisi*) and 40-70% in orange are reported (59).

Symptoms

Spreading decline appears in citrus orchards as groups of stunted or unthrifty trees with sparse foliage, small fruits, and retarded terminal growth. Leaves have a tendency to wilt during hot and dry periods, but may respond to moisture and show temporary recovery. New branches do not come out or their development is retarded. After some years the trees have a large number of drying or dead branches. The number, size and quality of fruits declines.

Symptoms of decline spread steadily to more trees each year. Examination of the root system reveals that below 50 cm of soil young feeder roots are considerably reduced in number or may be absent, which causes the above ground symptoms of starvation usually a year after root infection. New roots that develop are also destroyed by the nematode. Typical lesions are present on roots that have not been totally destroyed.

Morphology and disease development

The nematode, *Radopholus similis* (burrowing nematode), is described under banana root and rhizome rot. All stages of the male and female

can enter any part of the root and are found in root tissue as well as outside in the soil. The nematode spends its life and reproduces inside the cavities caused by destruction of cells in the root cortex where one life cycle is completed in 20 days. Although in banana *R. similis* does not enter the stele, in citrus it enters the stele through the endodermal passage cells. There, the nematodes accumulate in the phloem and cambium which they destroy in time and form nematode-filled cavities (58). In citrus roots a temperature of 25°-26° C is optimum for activity and development of the nematode (201). At this temperature one generation (egg to egg laying female) takes about 18-20 days.

The burrowing nematode can survive in sandy loam soil for 6 months in absence of the host roots. The life span depends on life span of the feeder roots which is shorter than the life time of the tree. In citrus orchards, the maximum numbers of the nematode are found between 0.3 and 1.8 meters but may be present as deep as 4 metres. The nematode is generally not found in the top 15 cm of soil.

Most of the spread from plant to plant is through root contact or near contact. The larvae migrate to a distance of several metres. However, long distance dispersal of the nematode and its introduction in an unaffected area occurs through transfer of infected planting stock. Once established in a new locality the nematodes can spread locally through root contact, soil, water and farm implements. Depending on the host the nematode can spread at the rate of 6 to 60 metres per year. Closeness of roots plays the major role in lateral spread. When roots of different trees are in close contact the lateral spread is fast. The average extent of spreading decline is 1.6 trees per year (202).

Management

Pull and treat method followed for control of spreading decline involves pulling out and destroying the infected trees and fumigating the soil. D-D was used for fumigation at the rate of 672 kg/ha. The land is either left fallow, keeping weeds out by herbicides, or such antagonistic crops as asparagus or marigold are cultivated for two years before replanting of citrus. To prevent introduction of the nematode in new areas through planting stock, bare rooted citrus plants are treated with hot water at 50° C for 10 min before planting.

■ NUTRITIONAL DISORDERS IN CITRUS

The normal growth of citrus trees requires nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, iron, zinc, manganese, copper,

boron and molybdenum as essential elements in their balanced nutrition. These elements are obtained by the tree from the soil. When one or more of these elements are in short supply, physiology of the tree is disturbed and aerial parts, especially leaves, show signs of hunger. Roots become weak and are ultimately dead. Loss of root functions weakens the absorption of minerals and water from the soil and nutritional deficiency symptoms appear on leaves and tender aerial parts.

The nutritional disorders show such symptoms as are commonly seen in the attack of fungal, bacterial, nematode and virus pathogens. Root rot, stunting, loss of green colour, and die-back are some such symptoms. Generally, these symptoms appear on grown-up or old trees when the demand for nutrients is high due to size of the tree. The nutritional deficiencies can be caused by any one or more of the following conditions: deficiency or loss of the particular nutrient in soil, leaching of the nutrient by irrigation and rains in light soils, non-availability of some elements such as manganese, zinc and iron due to excess of carbonates in the soil, loss of soil humus due to improper cultural practices, use of wrong combination of fertilizers and manures, defective rootstock and scion combination, poor irrigation, disturbed root and leaf functions due to attack of parasites and presence of hard pan below the root system. Before starting new orchards soil test for nutrients in soil and soil treatment against parasites are essential.

Nitrogen

Trees do not grow normally in nitrogen deficiency and remain stunted. New leaves remain small, narrow, and can easily detach. They are lighter green in colour. Old leaves are yellow. Such trees suffer from a certain amount of defoliation and look more open than normal trees. If the deficiency continues for a long time drying of some twigs may occur. Normally, the trees do not die but their productivity is decreased.

A soil having 250 kg/ha easily oxidized N can be considered N-deficient. Old leaves with less than 2% nitrogen (on dry weight basis) indicate N-deficiency in the tree (164). In these situations, nitrogen can be applied to soil around the tree as fertilizer or it can be sprayed as 2-5% urea. Foliar application needs several sprays during the year.

Phosphorus

Deficiency of phosphorus in the trees can be due to several reasons. Increased soil acidity hinders availability of phosphorus. This may happen if the soil contains sesquioxides of iron and aluminium. In heavy rainfall areas this nutrient is washed out from the soil. Signs of phosphorus

deficiency are more or less similar to those of nitrogen deficiency. Apical growth of the tree and of the root is stopped. Twigs are short and thin and grow in a straight manner. Older leaves show loss of shining green colour and sometimes they show greenish bronze colour. Some of the leaf veins are destroyed and lamina surface shows spots at such points. Older leaves shed early. Young or new leaves normally show the hunger signs late because they draw the nutrients from older parts. Lateral buds dry and fall. Flowering and fruiting is reduced. The skin of the fruit is thick and rough and the fruit becomes soft before ripening.

In neutral or alkaline soils phosphorus deficiency can be corrected by application of soluble phosphorus. In acidic soils insoluble forms of phosphorus are also effective. Annual test of soil for phosphorus content is essential because continuous use of phosphorus increases its level in soil above the required limit which hinders availability of zinc and copper.

Potassium

There are no reports of harm to citrus trees as a result of potassium deficiency. In potassium deficiency, apical growth of the tree stops. Young twigs remain weak and break off before becoming hard. Loss of green colour, gummosis of branches, rolling, crinkling and spotting of leaves may be due to potassium deficiency.

If leaves contain 0.2% or less potash (on dry weight basis), the element is deficient in the tree. For good sized fruits and normal skin this level should be 0.4 % (71). If potassium deficiency is suspected this nutrient can be supplied through potassium sulphate or potassium chloride. Light soils usually need more potash. Excess of potash in soil hinders availability of manganese, zinc and magnesium.

Calcium

The early sign of calcium deficiency in the tree is yellowish colour of leaves. The midrib looks more yellow than the nearest tissue. Leaves at the tip of twigs start yellowing from the tip and margins. On the yellowish surface brown spots appear. Wither-tip and root rot are also signs of calcium deficiency. Calcium deficient fruits are prone to easy attack of soft rot bacteria and fungi.

In the type of soil commonly used for citrus cultivation the chances of calcium deficiency are not much. In acidic soils there is less calcium and plants take in more potassium. Application of' lime or gypsum at the rate of 5-9 kg/ tree is recommended for correcting acute calcium deficiency (38).

Magnesium

Deficiency of magnesium causes interveinal chlorosis of leaves. The loss of green colour spreads all over the lamina surface except the tip and basal portion which remain green. Sometimes veins are thickened and become rough. Continued deficiency of the nutrient leads to defoliation and wither-tip.

Highly acidic soils have low magnesium level. In alkaline soils magnesium is present but due to excess of potassium and calcium it becomes unavailable to the tree. Excess of phosphorus also reduces availability of magnesium. Signs of magnesium deficiency may appear in nitrogen-deficient trees. Magnesium is required by the plant for chlorophyll synthesis and seed setting. Therefore, symptoms of magnesium deficiency become more conspicuous when fruiting has started and is more common in trees with seeded fruits. Since magnesium is highly mobile in the plant it gets accumulated in growing twigs and young leaves. Thus, deficiency symptoms are most prominent in old leaves. Deficiency of magnesium can be corrected by application 7-9 kg magnesium sulphate per tree or by spray of 2 % magnesium sulphate solution.

Sulphur

Generally this element is not deficient in plants or in the soil. Different inorganic fertilizers used in cultivation supply the desired quantity of sulphur. Symptoms of sulphur deficiency are similar to those of nitrogen deficiency. New leaves show the signs of deficiency early.

Boron

Boron is an important element for formation of the middle lamella. Generally, its deficiency is not seen in lime, oranges and sweet orange. It is rarely deficient in alkaline soils. In the light sandy (17) soils with acidic reaction and in the hills its deficiency has been noted in fruit trees such as mango and apple. In apple, boron deficiency is the cause of browning and decay of the core. In alkaline soils boron may be in excess and may cause boron toxicity. Symptoms of boron deficiency are aggravated under conditions of continued soil moisture deficiency.

Trees suffering from boron deficiency show yellowing of leaves starting from the tip and margins. Veins get thickened and corky on the lower surface of the leaf and may rupture. In mandarin oranges, leaf surface at the base of veins turns brown. Destruction of the vascular tissue is a typical effect of boron deficiency. As a result, plants show drooping and wilting even when soil contains enough moisture. Defoliation and

wither-tip follow this stage. In immature fruits, the inner white layer is thick. Due to rupture of tissue, gum exudation takes place which deposits as brown spots on the fruit. Such fruits fall down before ripening.

Excess of boron is toxic to plants. Before its application chemical analysis of soil and leaf tissue should be done to determine the quantity to be applied. Leaf tissue should have more than 25 ppm boron on dry weight basis. If less, boron can be applied as spray of boric acid (0.1%) or as soil application of borax (10-15 kg/ha).

Copper

Deficiency of copper in plants has been described under different names such as die-back and wither-tip, ammoniation, red rust, and exanthema. Cu deficiency occurs either due to lack of the nutrient in the tissues or due to disturbed balance between copper and nitrogen. Deficiency symptoms have been generally noticed when there is excess of nitrogen in soil. Roots of Cu-deficient plants fail to absorb zinc from the soil and zinc deficiency symptoms also appear.

The tender, long and pointed twigs of the tree twist to form a *S*-like structure. The leaves of these twigs are unusually long and deeper in colour. The midrib is slightly bent on the upper side. In acute deficiency of Cu, small leaves are formed on new twigs. They are mottled. Such leaves shed and the twig starts showing wither-tip. Old leaves are darker green and twisted. Blisters and cracks appear on the bark. Gum exudation occurs through these cracks. Brown spots may appear on skin of the fruit and they may also crack. The core of the fruit shows necrosis of tissue and gum deposit occurs in this area.

Normally, when copper fungicides are used to control gummosis and other fungal and bacterial diseases the deficiency of copper in the tree is not likely to occur. Otherwise, a mixture of 0.5% copper sulphate and 0.2 % lime should be sprayed. For soil treatment, 230 g of copper sulphate mixed with 115 g of lime can be applied per tree.

Zinc

Deficiency of zinc is described under different names in different countries such as frenching, leaf mottle and foliocellosis. Among the nutritional problems of plants, zinc is only second to nitrogen. In light soils in heavy rainfall areas zinc is washed out from the soil. In such areas zinc is deficient in the soil. In alkaline and heavy soils of high rainfall areas, zinc is not absorbed by the plants even if present in sufficient quantity in the soil. Attack of nematodes, shortage of humus, and unsuitable soil for the particular rootstock also cause zinc deficiency. The symptoms of Zn

deficiency are so pronounced that they change or mask the symptoms of other nutrient deficiencies. Zinc and manganese deficiency symptoms usually appear together. Deficiency signs of iron, copper and magnesium can also be present simultaneously.

The symptoms of Zn deficiency appear early on new twigs and leaves. The twigs remain stunted. Leaves are small with interveinal chlorosis. These foliar abnormalities give the tree a bushy appearance with erect branches. Symptoms are more conspicuous on the side facing the sun. Die-back is also a symptom of zinc deficiency.

Zinc deficiency is easily corrected by foliar spray of 0.4-0.6% zinc sulphate solution 2-3 times a year. Addition of lime (half the quantity of zinc sulphate) to the mixture avoids chances of phytotoxicity. Generally, corrective measures for zinc and manganese deficiency are taken together.

Manganese

Manganese deficiency is found in all regions growing citrus. In alkaline soils manganese is generally deficient along with zinc. In acidic soils the amount of manganese may be low. High pH of the soil hinders uptake of manganese by roots.

Manganese deficiency symptoms are similar to those of zinc but the size of leaves is not decreased and mottling is more pronounced on shady side of the tree. Loss of green colour is not uniform all over the lamina and symptoms do not appear immediately after formation of twigs. Die-back is not acute. Manganese deficiency can be corrected by spray of 0.4- 0.6% manganese sulphate.

Iron

Deficiency of iron is not a problem in citrus trees. In alkaline soils uptake of iron is low. Leaves are lighter green or yellowish. Against the yellowish background network of dark green veins is very conspicuous. As the leaves grow, they become thin and translucent and sometimes become almost white. Only the tissue adjacent to midrib remains green. The chlorotic appearance of leaves is more pronounced during winter and spring.

Although sprays of 0.4-0.8% ferrous sulphate give some relief from iron deficiency, no effective method is known. Avoidance or correction of conditions that hinder uptake of iron is advised. Excess of lime or calcium, moisture, phosphorus, zinc, copper, manganese, and microbial activities reduce availability of iron to plants.

Molybdenum

White or yellowish spots appear on leaves in molybdenum deficiency. These spots may coalesce. After sometime they may be masked. On the underside of leaves below the spots gum deposit is seen. In acute shortage of this element leaves fall down. Die-back of twigs also occurs. Ammonium or sodium molybdate is sprayed to correct this deficiency.

From time to time, nutrient mixtures have been developed and recommended for use in orchards to avoid nutrient deficiencies. Such mixtures may prove harmful if one or more particular elements are not deficient and their addition through the mixture may harm the trees.

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