# DISEASES OF UNDETERMINED ETIOLOGY

## Studies on the Cause of the Fruta Bolita or Declinamiento Disease of Citrus in Argentina

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At the 5th IOCV Conference, Pujol *et al.* (1972) reported on a decline called fruta bolita (FB) or declinamiento that affected sweet orange trees grafted on trifoliate orange in the Province of Misiones, Argentina. At that time, the disease had spread to an estimated 10-20 per cent of the 6,000 ha of sweet orange on trifoliate stock. Now, 10 years later, nearly all sweet orange trees on trifoliate orange stock are either unproductive or have been pulled because of FB decline.

In September 1976, an Argentinian-German citrus project was started to study the FB problem and the project became fully functional in 1978. The first results of studies on the FB problem by members of this project and other cooperating research workers are reported here.

### SURVEY, EXPERIMENTS AND RESULTS

**Survey on the geographical occurrence of FB in Argentina.** A survey on the presence of FB was carried out in Misiones and other provinces of Argentina. The diagnosis was based on the major symptoms: decline, small fruits and zinc deficiency in leaves. As these symptoms are not specific, errors in identification are possible; however, all trees were inspected for other problems that might cause FB-like symptoms, such as psorosis, root rot, rodent attack, waterlogged soil, etc.

In Misiones, FB symptoms were found in plantations of sweet orange on trifoliate stock of all ages. The disorder now affects all sweet orange cultivars, although differences between the cultivars were noted earlier (Pujol *et al.*, 1972). The youngest plantation in which FB symptoms were found was 2 years old; the oldest was more than 45 years of age. The disease has spread from orchard to orchard independently of cultural practices or soil type. In contrast to the situation earlier (Müller *et al.*, 1974), very few outstanding trees were found in severely affected orchards.

Although originally FB was mainly a problem in the combination of sweet orange on trifoliate orange, now severely affected 8-10-year-old orchards of the combinations of sweet orange on Rangpur lime and sweet orange on rough lemon can be found. It appears that the stress caused by the severe droughts in the years 1976/77 and 1977/78 speeded up the appearance of FB symptoms in less susceptible combinations. In one orchard of sweet orange on Rangpur lime, the percentage of trees showing FB symptoms increased from 10 per cent in 1977 to about 80 per cent in 1978. The most tolerant rootstock appears to be Cleopatra mandarin. Grapefruit, lemon, and mandarin scions on trifoliate orange rootstock do not appear to be affected. With few exceptions, the inspected orchards were in poor condition and had not been properly fertilized or sprayed. The soils in some orchards — mostly red lateritic soils — have a very high aluminum content. Virus diseases are common, especially psorosis. Exocortis and gum pocket disease (trifoliate pitting) were common before trifoliate orange was abandoned as a rootstock. Tristeza stem pitting is common on grapefruit and in one orchard, clear symptoms of cristacortis were found on Valencia: deep vascular pits penetrating into the wood and crestlike growth of the bark. Electron microscopic studies of sections cut from peduncles of grapefruit in one orchard showed the presence of tristeza particles and particles of a spherical virus which gave a positive reaction with citrus leaf rugose virus antiserum obtained from S. Garnsey (R. Casper, personal communication).

Typical FB symptoms were found in sweet orange trees on trifoliate orange rootstock in the Bella Vista and Saladas area of Corrientes Province, in the Libertador San Martin area of Jujuy Province and in the Oran area of Salta Province (Schwarz and Monsted, 1976*b*). As the major rootstock in these provinces is Cleopatra which is tolerant to FB, it is not known how far FB has spread from the foci. In one orchard, next to a severely affected sweet on trifoliate orange orchard, FB symptoms were observed in sweet orange on Troyer citrange.

Declining sweet orange trees on trifoliate orange, found in the area of Concordia, Entre Rios Province (Schwarz and Monsted, 1976*a, c*) showed symptoms résembling marchitamiento repentino (Salibe *et al.*, 1976) rather than those of FB. Affected trees were found to occur only on patches of heavy clay-like soil and the disease did not appear to spread.

**Reaction of FB-infected sweet orange (Calderon) grafted on different rootstocks.** In a first trial, Calderon sweet orange buds from FB-infected trees on trifoliate orange (Misiones) were grafted to three seedlings each of 33 rootstock cultivars in October 1977 and the same number were grafted with Calderon buds from Entre Rios, a province in which FB does nòt occur. The material from Misiones was thus infected with FB and tristeza, whereas the material from Entre Rios was infected only with tristeza virus.

The rootstocks used were: trifoliate orange (six cvs.), trifoliate orange hybrids (seven cvs.), rough lemon (three cvs.), sweet orange (three cvs.), Rangpur lime, Cleopatra mandarin, Orlando tangelo, Mexican lime, Indian lime, sour orange, Dweet tangor, grapefruit, pummelo, nasnaran, nanshodaidai, and Volkamer lemon.

So far, no clear differences between the scions from Misiones and Entre Rios were noticed. In both groups, the combinations of sweet orange on Mexican lime, sour orange, grapefruit, and pummelo showed severe stunting and strong deficiency symptoms caused by tristeza. In both groups, the take on trifoliate rootstock was poor and stunting was common. These symptoms were especially severe in scions on Yuma citrange and Sacaton citrumelo rootstocks. The latter hybrid performed poorly in the presence of "normal" Brazilian tristeza strains (Salibe *et al.*, 1972).

In a second trial, 16 seedlings of each of 22 of the rootstocks mentioned were grafted with Valencia material from FB-affected trees. Five months after budding, marked differences in the length of the shoots were noticed. The shoots of the sweet orange grafted to trifoliate orange stock were weakest, with an average length of 6-12 cm.

The best growth occurred on nanshodaidai (29 cm), Empress mandarin (28 cm), and sweet oranges (25-37 cm). Scion growth on rough lemon (19-21 cm) and Rangpur lime (14 cm) — normally the most vigorous trees — was rather poor. Although these early results may not be indicative of the performance of these combinations in later years, it is interesting to note that, as in FB-infected orchards, sweet orange on sweet orange and mandarin grow well, and sweet orange on trifoliate orange grows poorly.

**Scion-rooting, approach-grafting and double-scion trials.** When visiting scientists and local research workers expressed the opinion that FB had many features of tristeza (Schwarz and Valiela, 1969; Rossetti *et al.*, 1970; Müller *et al.*, 1974), a small trial was initiated in 1969 by members of the extension service in Montecarlo to investigate whether, as in the case of tristeza, FB-affected sweet orange trees on trifoliate orange would recover when the scion was approach-grafted with a tolerant variety (mandarin) or when the tree was scion-rooted.

Seven trees of Calderon sweet orange on trifoliate orange with early FB symptoms were approach-grafted with three Cleopatra mandarin seedlings each. Five FB-affected trees of the same combination were scion-rooted by cutting three windows of 2x5 cm in the bark of the scion trunk near the bud union and then covering the base of the trunk with soil. Five trifoliate orange seedlings were budded at the same time with Calderon sweet orange and with Eureka lemon. In the 10 years between treatment and inspection, the trees were neither fertilized nor sprayed and they also had suffered from two successive years of drought. It was, however, clear from the performance of the trees — when compared with that of untreated controls which were in an advanced state of decline (fig. 1) — that the scion-rooted trees (fig. 3) as well as the trees approach-grafted with Cleopatra mandarin (figs. 2, 4, 5) had recovered. The trifoliate rootstocks of the approach-grafted trees and the scion-rooted trees had died. In one case, where only one of the approach-grafts had taken, only the approach-grafted sweet orange branch recovered, whereas the rest of the sweet orange scion and the trifoliate orange rootstock had declined (fig. 5). In 1973, a commercial company scion-rooted about 200 trees (Valencia on trifoliate orange stock) after about 10 per cent of the trees had started to show FB symptoms. Now, 6 years later, the scion-rooted trees have recovered and no new cases of FB have been recorded.

Only the lemon scions developed normally on the double-budded trifoliate stocks, whereas the sweet orange scions developed poorly and died back in the first five years after grafting.

**Physiological studies.** *a.) Accumulation of zinc and phenolics in the outer layers of trunk wood.* Following the techniques used by Wutscher *et al.* (1977), holes (1.3 cm wide and 2.5 cm deep) were drilled on both sides of each trunk 10-20 cm above the bud union. After the bark was removed, chips of wood were collected for analysis of water soluble phenolics, Zn, Mg and K.

The results of the analyses of samples from FB-affected and normal-looking trees showed (table 1) that — as with blight (young tree decline) (Smith, 1974; Wutscher *et al.*, 1977; Wutscher and Hardesty, 1979) — the samples from FB-affected trees contained clearly higher levels of Zn, phenolics and somewhat higher levels of K and Mg. There were, however, exceptions. In orchards 5, 6 and 7, normal-looking trees showed increased Zn and phenolic contents. Experience with blight (YTD) in Florida and with "marchitamiento repentino" in Entre Rios has shown that trees with elevated Zn levels in the trunk wood tend to show visual decline symptoms within 2 years. Normal-looking sweet orange trees on Rangpur lime and rough lemon rootstock with abnormally high levels of Zn and phenolics may be in an incipient stage of decline. Observations in orchard no. 5, where, after a drought, the incidence of trees showing symptoms has increased from about 10 per cent to 80 per cent in 1 year, support this assumption.

*b.) Water uptake and dye uptake studies.* Water uptake of FB-infected





**Fig. 1. Calderon sweet orange on trifoliate orange stock showing severe fruta bolita symptoms (untreated control).**

**Fig. 2. Recovery of fruta bolita-affected Calderon sweet orange on trifoliate orange stock after approach-grafting with Cleopatra mandarin.**

**Fig. 3. Scion-rooted fruta bolita-affected Calderon sweet orange on trifoliate orange stock.**

**Fig. 4. Fruta bolita-affected Calderon sweet orange on trifoliate orange stock approach-grafted with Cleopatra mandarin; trifoliate stock shows dieback.**

TABLE 1
CONTENT OF WATER SOLUBLE PHENOLICS, ZN, K AND MG OF TRUNK WOOD OF TREES SEVERELY AFFECTED BY FRUTA BOLITA (S) AND NORMAL-LOOKING (N) TREES.*

| Orch. | Scion/ | Age | No. of | $\bar{X}$ Water soluble phenolics (mg/g)† | | Zn (ppm)† | | K (per cent)† | | Mg (per cent)† | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | stock | (yrs) | trees | N | S | N | S | N | S | N | S |
| 1 | Sw. O./Trif. | 23 | 4 | 4.2±0.2 | 7.5±1.0 | 4.7±1.0 | 10.5±1.5 | .205±.015 | .262±.028 | .038±.003 | .047±.004 |
| 2 | Sw. O./Trif. | 11 | 3 | 3.0±0.5 | 6.8±1.7 | 3.0±0.0 | 9.0±1.6 | .212±.017 | .270±.020 | .038±.001 | .053±.005 |
| 3 | Sw. O./Trif. | 8 | 7 | 3.4±0.5 | 7.9±1.2 | 3.0±0.8 | 9.0±3.0 | .173±.016 | .291±.061 | .037±.006 | .049±.011 |
| 4 | Sw. O./Trif. | 7 | 3 | 3.3±0.2 | 6.3±0.5 | 3.0±0.0 | 9.7±3.0 | — | — | — | — |
| 5 | Sw. O./Rang. | 10 | 4 | 6.1±2.4 | 7.8±2.8 | 15.5±8.9 | 11.7±4.8 | .219±.025 | .210±.020 | .044±.010 | .052±.014 |
| 6 | Sw. O./Rang. | 6 | 3 | 5.5±1.2 | — | 7.7±2.9 | — | .163±.016 | — | .038±.000 | — |
| 7 | Sw. O./Rough | 6 | 3 | 6.1±0.7 | — | 10.0±2.2 | — | .234±.032 | — | .040±.005 | — |
| | | | | H | B | H | B | H | B | H | B |
| Means from 19 blight-affected (B) and healthy (H) trees of sweet orange/rough lemon in Florida‡ | | | | 3.5 | 5.7 | 4.0 | 22.0 | .226 | .336 | .031 | .049 |

*Location: Montecarlo, Misiones, Argentina.
†Means and standard deviations from means.
‡See Wutscher *et al.*, 1977.

and normal looking trees was measured by gravity injection (Cohen, 1974). A calibrated water container was connected with a plastic tube to a tubing connector firmly inserted into a 10x40 mm hole drilled into the trunk 15 cm above the bud union. The results of the water uptake trials (table 2) showed that the water uptake of FB-infected trees is clearly reduced when compared with that of healthy-looking trees. In general, the results are similar to those obtained with blight (YTD) (Cohen, 1974; Young and Garnsey, 1977; Young *et al.*, 1978).

Dye injections into diseased trees showed that the movement of the colored solutions was limited to a very restricted area above and below the injection site (fig. 6). There appeared to be little lateral movement of the dye solution. Thus, the reduction in water movement indicated by color patterns in trunk sections of infected trees is similar to those found in blight-affected trees (Young and Garnsey, 1977).

**Chromatographical studies of phenolics extract of FB-affected and normal trees.** Because FB-affected trees show a higher content of water-soluble phenolics in the trunk wood, it appeared possible that, as in the case of citrus greening (Schwarz, 1968), specific markers might be found in the bark of the scion or rootstock of FB-affected sweet orange on trifoliate orange stock.

Thirty FB-affected and 30 apparently healthy trees were sampled in Misiones and 30 apparently healthy trees in Concordia, Entre Rios. Three 1-gm samples


**Fig. 5. Cleopatra mandarin seedling (right) approach-grafted to Calderon sweet orange branch growing out from fruta bolita-affected sweet orange on trifoliate orange stock. Both scion and trifoliate stock show dieback from FB decline; only the approach-grafted branch grows normally.**

**Fig. 6. Staining pattern of color-injected sweet orange trunk on trifoliate orange affected by fruta bolita. Strong pattern is 6 cm above injection site 1. Only single vessels showed stain 25 cm above injection site 2.**

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TABLE 2
WATER UPTAKE BY TRUNK INJECTION ABOVE THE BUD UNION OF NORMAL-LOOKING (N) AND FRUTA BOLITA-INFECTED TREES (M = MODERATE, S = SEVERE SYMPTOMS).*

| Orch. No. | Scion/ stock | Age of trees (yr.) | No. of trees | m1 water uptake/hour† | | |
|---|---|---|---|---|---|---|
| | | | | N | M | S |
| 5 | Valencia/rough lemon | 11 | 10 | —— | 11.6±3.5 | 2.3±1.2 |
| 3 | Calderon/Trif. | 8 | 8 | 20.6±7.3 | 13.3±3.4 | —— |
| 8 | Calderon/Trif. | 8 | 13 | —— | 6.5±1.8 | 2.4±1.2 |
| 9 | Calderon/Trif. | 7 | 10 | —— | 13.7±7.4 | 2.2±0.6 |
| 10 | Calderon/Trif. | 8 | 7 | —— | 12.6±5.6 | 4.1±1.2 |
| 11 | Calderon/Trif. | 8 | 7 | —— | 7.0±1.7 | 1.8±1.0 |
| 12 | Calderon/Trif. | 7 | 10 | —— | 10.9±3.8 | 1.9±0.7 |

*Location: Montecarlo, Misiones, Argentina
†Means ± standard deviations.

were taken 10 cm below the bud union on three sides of the trunk of the trifoliate orange stock, and three 1-gm samples were taken from 2-year-old wood from the sweet orange scion of each tree. The three samples of the scion and stock of each tree were pooled, cut into small pieces, and shaken for 1 hour with enough water to cover them. The solution was then concentrated by evaporation, spotted three times on a silica gel TLC plate and developed with water-saturated n-butanol following the method described by Schwarz (1968).

A comparison of the fluorescent pattern of the sweet orange scion bark extracts from normal-looking trees (Misiones and Concordia) and the samples from FB-infected trees (Misiones) showed no clear differences.

The bark extract pattern of trifoliate stock of some orchards in Concordia and Misiones — independent from their infection with FB — showed a series of bright fluorescent spots, the main marker being a violet blue spot with an RF of 0.74. Similar patterns were found in trifoliate bark extracts from trees infected with exocortis (Schwarz, 1968) and in trifoliate bark extracts of trees infected with gum pocket (Schwarz, unpublished, and Feldman, personal communication, 1968). A chromatographical analysis of trifoliate bark samples from trees infected with exocortis and gum pocket obtained from J. L. Foguet, Tucumán, showed that both diseases produce fluorescent markers which are similar to those found in the studies mentioned above. It thus appears possible that the fluorescent marker pattern in the trifoliate stock bark extracts is indicative of a latent infection with exocortis or gum pocket. None of the trees showing these patterns showed visible symptoms of either disease.

The greening-marker, gentisic acid glucoside, reported by Mello *et al.* (1974) in the albedo of FB-affected fruits was not found in any of the samples tested.

**Histology.** Bark samples taken across the bud union of FB-affected and normal-looking sweet orange trees on trifoliate orange stock were fixed in FAA solution progressively stained with hematoxylin and counterstained with resorcine blue. Some samples were sectioned and stained by Dr. H. Schneider.

FB-infected samples showed 350 to 500≢ of functional phloem above the bud union with areas of callose formation. Below the bud union, there were heavy deposits of callose, sieve-tube necrosis and collapse. There were also excessive amounts of fibers and hyperplastic rays; formation of callus tissue was not observed.

The samples processed by Dr. Schneider and our samples from different orchards showed the same symptoms.

These histological changes are similar to those found above and below the bud union of tristeza-infected sweet orange on sour orange stock.

In addition to the phloem symptoms, the trifoliate bark of the FB-infected combination sweet orange on trifoliate orange showed gum-impregnated patches. In root samples of FB-affected trees, a large number of "plugs," associated with YTD infection by Childs (1965), were found (Childs, personal communication, 1978).

## DISCUSSION

The results show that FB has properties of blight (YTD) and tristeza.

Both FB- and blight-affected trees show reduced water uptake (indicating xylem blockage) and increased levels of phenolics, Zn, and to a lesser extent K and Mg in the outer zone of the trunk wood. The accumulation of Zn and phenolics appears to be the most reliable diagnostic tool to identify the disease. This diagnostic technique may be especially useful for early diagnosis of FB in commercial trees in rootstock trials.

Tristeza-affected sweet orange trees on sour orange rootstock absorb the same amount of water as healthy trees (Cohen, 1974; Wutscher *et al.*, 1977). These results have been obtained for trees infected with the rather mild Florida tristeza complex. It would be interesting to determine if more severe tristeza complexes like the Capão Bonito strain of tristeza in Brazil or the tristeza complex found in Asia induces a blockage of xylem and buildup of phenolics and Zn in the trunk wood. The Capão Bonito tristeza complex, like FB, induces a severe stunting of Hamlin sweet orange on trifoliate orange. Hamlin trees on Rangpur lime also show only moderate growth, whereas Hamlin trees on Orlando tangelo, sweet orange, and mandarin stocks grow vigorously (Müller and Costa, 1975). The Capão Bonito strain of tristeza, however, causes pitting on sweet orange and Rangpur lime, a symptom not associated with FB.

On the other hand, FB, like tristeza, produces phloem necrosis below the bud union, dieback of roots, bud-transmissibility, and a rapid spread of the disease in the field, which are indicative of a winged vector — features not associated with blight (YTD).

All three diseases, FB, tristeza, and blight, affect only certain scion/stock combinations, although the sensitive/tolerant combinations are different for tristeza. Trees affected with FB, tristeza and blight recover when they are scion rooted. FB- and tristeza-affected trees also seem to recover when they are approach-grafted with tolerant stocks, although the original status of such trees may be questionable.

In tristeza-affected sensitive scion/stock combinations, only the phloem below the bud union becomes necrotic, whereas the xylem shows no histological changes. In trees affected with blight, only the xylem above and below the bud union is blocked, whereas the phloem is normal. In FB-affected trees, on the other hand, phloem necrosis occurs below the bud union and xylem blockage occurs above and below the bud union. Thus the chances for a successful recovery by approach grafting and scion rooting of affected trees should be better for tristeza-affected trees than for trees affected by either FB or blight. With the latter disorders, approach-grafting and scion rooting should be applied as early as possible at a stage before the xylem is blocked.

Further studies on the physiological effects of FB, various tristeza complexes, and blight are needed to define the relationships among these diseases.

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