PLANTING & MAINTAINING HUANGLONGBING (GREENING) FREE ORCHARDS IN SOUTHERN AFRICA

H.F. LE ROUX & C.H. BUITENDAG
Citrus Research International

Abstract
Huanglongbing (HLB) remains one of the most dangerous citrus diseases in South Africa. However, since the late 1980s, the industry has developed strategies which have succeeded in controlling the advance of greening and ensuring the continuation of profitable citrus production in areas threatened by the disease. These strategies included the provision of HLB-free nursery trees certified by the South African Citrus Improvement Programme, a reduction of the inoculum through an ongoing programme of removing plant parts showing HLB symptoms and the use of systemic insecticides applied as trunk applications to control the vector, Triozia erytroae (Del G.). This led to the reintroduction of citrus to areas previously abandoned because of HLB. Orchards well managed are performing well. However, a danger exists where HLB may emerge once again as a result of a new generation of growers and consultants not being aware of the consequences of the disease. The interval provided by the development of effective measures to control the vector was not effectively utilized in order to find long-term solutions.

Introduction
Huanglongbing (HLB), better known in South Africa as citrus greening, has had a devastating effect on citrus production in Mpumalanga, Limpopo, Gauteng and NorthWest Province and KwaZulu-Natal since it was first observed in 1928 (11). It has since spread to certain areas of the Western and Eastern Cape (8). The disease is caused by an uncultured phloem-restricted gram negative bacterium belonging to the subdivision of the Proteobacteria known as Liberibacter africanum. Another species, L. asiaticum which can be found in other parts of the world, e.g. Asia, does not occur in Africa (6).

Buitendag (2) divided the development and spread of HLB in South Africa broadly into three periods:

The period prior to 1965, before it was established that the citrus psylla, Triozia erytroae Del G., was the vector of the disease. During this period the disease was observed, but it was identified as a deficiency symptom with zinc deficiency as the main cause.

In these years the disease spread rapidly to the areas where citrus psylla was endemic. Its subsequent spread to other parts of the country resulted from the movement of infected nursery
trees. In the absence of a correlation between psyllid presence and greening, no steps were taken to control the spread of the disease.

In the period subsequent to 1965, during which it was discovered that the citrus psylla was indeed the vector of the greening disease, it was realized that control of the vector could prevent spread of the disease. However, lack of suitable treatments reduced grower ability to effectively protect new growth from psylla attack. The emphasis was rather placed on the ‘healing’ of sick trees with the aid of antibiotic injections and only heavy infestations of citrus psylla were controlled. The general suppression of citrus psylla was further neglected as a result of the decline in usage of organophosphates following the development of resistance by the red scale, Aonidiella aurantii (Mask.) to these materials and resulting grower need to favour the biological control of the pest.

Foliar sprays to control citrus psylla during this period also had a disadvantage of not having a long enough residual effect. One spray, for instance, could not protect a growth cycle completely against psyllid breeding. For this reason more than one foliar spray (even up to three sprays) had to be applied per growth cycle for complete control. Such a drastic spray programme for the control of citrus psylla alone was very detrimental to the biological control of red scale, and was also expensive. This resulted in high populations of psyllids being tolerated in citrus orchards, and the subsequent unhindered spread of greening disease. At the same time it was found that the antibiotic injections were only able to give temporary relief from greening and could not heal trees completely.

During the period subsequent to 1970, the number of citrus orchards in the areas where citrus psylla was endemic, decreased rapidly as a result of the disease. Virtually no new plantings were established, and where single orchards were planted, young trees soon became infected by the disease. During the latter part of the eighties, much more attention was given to the control of the greening problem. It was accepted that greening control is directly related to the control of psyllid numbers in citrus orchards. In response to this, more effective chemical programmes were developed to control citrus psylla, and supplementary cultural treatments were also devised. This was combined with the use of healthy nursery trees and the removal of infected branches in order to reduce the inoculum.

During the mid-1970s citrus production was virtually eliminated in three major citrus areas and it was estimated that 4 million of the 11 million trees in South Africa were infected (3). It was predicted that with the exception of the Eastern and the Western Cape citrus production areas, citrus production in South Africa would be eliminated. To the contrary, citrus production increased to approximately 55 000 ha or 25 million trees in 2002.

The fourth era was during the mid to late nineties. During this time the disease was detected by the University of Pretoria in the Western Cape. This was confirmed by both Bové (INRA) and Cronje (SASEX) using PCR detection. It was shown that greening could be found from Stellenbosch, beyond Somerset West, Grabouw, Hemel-en-Aarde Valley up to Buffelsjagsrivier. It was also detected at Amanzi in the Eastern Cape.

The year 2000 introduced the fifth era. The northern areas came out of a dry cycle. Good rains with cool weather existed and an increase in psylla populations occurred. Psylla control was neglected and thousands of trees became infected. This resulted in the largest removal of orchards during 2002 as a result of greening since the 1970s. This article discusses the strategy used to control the advance of greening and ensures the profitable continuation of citrus production in areas threatened by the disease. In order to achieve this growers are reminded to refer to the disease triangle (Fig. 1). Should the removal of any one of the three factors involved in the triangle occur, no disease can take place. This will be the starting point to discuss the planting and maintenance of HLB-free orchards in South Africa.
Though *L. africanum* can survive in a few Rutaceae species other than Citrus, citrus has always been the main source of inoculum. As late as the 1970s, nurserymen in areas where HLB was endemic were still cutting budwood from infected orchards and grew trees without proper control of citrus psylla. This not only spread the disease within the area of production, but also to areas which were initially disease-free.

The phenomenon changed with the formation of the Citrus Improvement Programme in 1976. Trees from all the different citrus cultivars used in South Africa were selected for production, after which budwood from these trees was taken through shoot-tip grafting to ensure that they were disease free. The budwood was then inoculated with a mild strain of Tristeza virus and sent to the Outspan Foundation Block (OFB), now known as the Citrus Foundation Block (CFB) for multiplication. The CFB is located in an HLB-free area in the Eastern Cape. Any new material brought into South Africa follows the same procedure. All the accredited citrus nurseries in South Africa participate in the scheme and order their budwood from the CFB.

Nurseries in an endemic citrus psylla area where greening is present are enclosed with psyllid-proof screening with double entry doors, and special shoots to remove trees from the inside to the outside of the nursery. In non-endemic areas, nurserymen are taking precautions against psyllid outbreaks. Upon observation of a single psyllid, the entire nursery is treated for the pest. Yellow traps can also be used in the nurseries to monitor adult psyllids. Nurseries are visited regularly by personnel from the CDIP who audit the Phytophthora status in the nursery. During these visits the trees are inspected for psylla as well.

Finally, the success of producing HLB-free trees lies within the certification of trees which are produced under psylla-free conditions in accredited nurseries. Unfortunately the certification of trees was terminated when the industry experienced difficulties during the past few years. It is hoped that the certification will once again soon commence.

The discovery of tolerant selections of the different cultivars against HLB would be of great importance to the industry. Despite several promising selections being screened over several years, to date none have been commercialized. However, the ITSC in Nelspruit has introduced a new project whereby embryo rescuing is used to retrieve healthy tissue from chimeras otherwise infected with greening, from which they endeavour to breed greening-tolerant citrus.

To restrict the movement of diseased material into disease-free areas, a law was proclaimed to prevent citrus planting material from being moved from HLB infested areas into the Cape Provinces. With the exception of Amanzi, this prevented the Eastern Cape from becoming infected, at the same time postponing the spread of the disease to the Western Cape by almost twenty years (4). The spread of the disease could have been slowed with an awareness programme using roadside signs on the main traffic routes to the Cape. New regulations with regard to the movement of citrus trees are currently being processed.
restricted, uncultered bacterium known as Liberibacter. The African species is known as L. africanum and is transmitted by the psyllid insect vector, T. erytreae, whereas the Asian species, L. asiaticum is transmitted by the psyllid, Diaphorina citri. Liberibacter multiplies both in the hemolymph and within the cells of the salivary glands of the psyllids (17). Though both psyllids can transmit both Liberibacter species the geographic distribution of the psyllids are such that D. citri transmits L. asiaticum and T. erytreae, L. africanum in nature.

During the 1970s tree injections using tetracycline hydrochloride were developed and applied commercially (13). However, it was not sustained as a commercial treatment because the method proved expensive, remission was only temporary, treated trees were inclined to produce small fruit, phytotoxic effects occurred at the injection site and high levels of residues were found in the fruit of treated trees. Some of these problems were resolved through the application of N-pyrrolidinomethyl tetracycline (PMT), by means of trunk injections with syringes (3). The antibiotic injections were, however, only able to give temporary relief from HLB and could not heal trees completely. Today no antibiotics are applied at all.

Another method used to reduce the inoculum is the removal of symptomatic plant tissue from infected trees. This in turn reduces the extent of HLB transmission by the vector (7). According to Buitendag and von Broembsen (3), trees can be divided in two age groups for the purpose of inoculum reduction.

(i) Trees up to 10 years of age: The recommendation is that when trees up to 5 years of age show symptoms of greening in the canopy, they should be removed. Trees from 6 to 10 years old, which are 50% or more infected with greening should also be removed. Where the infection is lower than these thresholds, only the infected branches should be removed. Interplanting young trees in orchards older than 10 years which have a history of greening is not recommended.

(ii) Trees older than 10 years: Individual branches infected with greening do not bear healthy fruit and will not recover. In addition, they consume the nutritional reserves of the tree. In the cases of trees with up to 40% of the branches infected, such branches should be marked and removed.

We are presently of the opinion that the best method of pruning a greening-infected tree is to do so with the aid of a tractor and chain - roots and all.

Psylla is attracted by new growth. Pruning stimulates new growth which, in turn, attracts psylla particularly on the cooler side of the tree. This re-growth must be protected against psylla infestations. If not, pruning is futile. New plantings in greening areas should preferably be designed so that hedging and topping is kept to a minimum. Trees which are to form hedgerows should be orientated in a north/south direction to minimize the creation of a suitable habitat for psylla breeding (1).

VECTOR

The reduction of the inoculum by infected plant parts and the use of greening-free plant material became two of the most important pillars of the HLB control strategy in South Africa. There was, however, one further pillar of importance - the control of the vector.

Diaphorina citri is absent in South Africa but not Trioza erytreae. During the 12th IOCV Conference in India a number of key features of the vector which necessitated being taken into account in the formulation of control strategies were summarized by Buitendag and von Broembsen (3), namely:

- While psylla are considered to have weak dispersal powers, they can move a distance of at least 1.5 km with wind (13). However, the insects are excellent invaders and can readily locate isolated areas of growth flush over several hundred metres (12).
- There is continuous movement of psylla between citrus and adjacent indigenous vegetation, when present. Indications are that by eradicateing native host plants from the vicinity of citrus orchards, psylla populations can be reduced in those orchards (16). Transmission of HLB from indigenous plants is another matter as Liberibacter could not be found in the indigenous Rutaceae plants tested in the Nelspruit Botanical garden over a period of two years. It was also shown that the Liberibacter found in the Cape Chestnut was not the same as those found in citrus.

- Adult citrus psylla are almost exclusively confined to young leaves and if new flush is present the vector will remain on the same tree for several hours, especially at night (15).
- The adult psylla cannot survive longer than 55 hrs away from suitable foliage, and the maximum lifespan in the absence of their host plants is estimated to be 85 hrs (15).
- Females prefer soft leaves for oviposition, thus influencing the cultivar as well as time of year of greatest reproductive activity (16).
- There is evidence that females can transmit greening to the plant during oviposition. Psylla nymphs emerging from the eggs may be infected with greening. This suggests that adult psylla may already be infected with greening without any acquisition feeding (17).
- Adult citrus psylla are able to acquire the greening organism within 24hrs of feeding on infected foliage and can transmit the
disease 24hrs after the acquisition feeding (17).

- Although contact and systemic insecticides may not prevent the spread of greening to an orchard they may serve to reduce the spread of the disease within the orchard (15).

The industry realized that control should be aimed at keeping psylla populations low in all orchards, thereby limiting their dissemination and that of greening to a minimum. However, it was found after the development of organophosphate resistance in red scale, that multiple sprays aimed at maintaining low populations of psylla throughout the spring, summer and autumn periods were impractical and caused pest repercussions. It was then realized that systemic treatments targeted specifically at psylla and applied either to the stem or to the roots of the tree would be most effective (3).

In 1977 a method for controlling psylla through the application of dimethoate together with irrigation water to the basin of trees was developed (14). For various reasons including high costs, erratic uptake due to different soil types, root health factors, the incompatibility of certain rootstocks with dimethoate and the possibility of accelerated microbial degradation of the soil-applied chemical occurring, this method subsequently fell out of favour (3).

Aldicarb (Temik®) was also applied to non-bearing trees. It moves systemically into the foliage and has a long residual action against psylla. As a result of the high dosages and frequent applications, the development of accelerated degradation of aldicarb by soil micro-organisms was common. In replant situations where the citrus nematode, Tylenchulus semipenetrans occurred, this rendered aldicarb ineffective as a nematicide (9).

In 1986 Buitendag produced a new breakthrough with the registration of monocrotophos (Azodrin® 40) as a systemic trunk application. Treatment costs using this chemical were relatively low, minimal disruption of beneficial insects occurred and its application using the Calibra trunk applicator was both easy and effective.

Control for 26 days was achieved using monocrotophos. The product also controls aphids, budmite, leafhopper and thrips on young flush. Unfortunately monocrotophos is due to be withdrawn from the market in South Africa at the end of March 2003. During the early 1990s, Buitendag developed a second chemical for use as a systemic insecticide by means of trunk applications, viz. methamidophos (Cirimite®).

This chemical was intended to provide an alternative if psylla developed resistance against monocrotophos. Unfortunately, methamidophos was only effective against psylla for 18 days. While this control period is relatively short, it is accompanied by a low residue level of the chemical in the tree and thus a short safety period between applications and harvest. Methamidophos was also found to be effective against certain mites, orange dog and thrips (3).

Imidacloprid (Confidor®) and acetamiprid (Mospilan®) were also tested. These are not organophosphates and thus reduce the likelihood of psylla developing resistance to the trunk-applied products. The residual effect of these products in the tree after trunk application is approximately twice that of monocrotophos or methamidophos respectively. The safety period between application and harvest is therefore also longer (3).

Trunk applications for monocrotophos and methamidophos can be used at the rates given in Table 1. When the trunk circumference exceeds 450 mm (140 mm diameter) these treatments become uneconomical and mevinphos or methamidophos foliar sprays should be used (1).

Table 1: Monocrotophos and methamidophos dosage rate in relation to tree-trunk circumference

<table>
<thead>
<tr>
<th>Trunk circumference (mm)*</th>
<th>ml monocrotophos 40 per tree when applied by applicator (undiluted)</th>
<th>ml methamidophos per tree when applied by applicator (undiluted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.1</td>
<td>0.08</td>
</tr>
<tr>
<td>40</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>50</td>
<td>0.3</td>
<td>0.25</td>
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<tr>
<td>100</td>
<td>0.8</td>
<td>0.8</td>
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<tr>
<td>150</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>200</td>
<td>2.8</td>
<td>2.0</td>
</tr>
<tr>
<td>250</td>
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<tr>
<td>300</td>
<td>6.5</td>
<td>6.0</td>
</tr>
<tr>
<td>350</td>
<td>10.0</td>
<td>8.0</td>
</tr>
<tr>
<td>400</td>
<td>15.5</td>
<td>12.0</td>
</tr>
</tbody>
</table>

*Measured at the narrowest part of the main trunk.

However, trunk applications do not only have advantages. Danger exists if used incorrectly or when formulations not registered for stem applications are used. It is of extreme importance that trees should not be treated when under stress. The stomata should be open and there has to be sap flow when treatments are done. If not, the products can become phytotoxic and can cause injuries to the trunks which, in extreme cases, could result in ring barking and ultimately the death of the tree.

DISCUSSION AND CONCLUSIONS

The use of disease-free planting material certified by the CLP, grown in psylla-free nurseries, the use of systemic insecticides applied to the stems of citrus trees to control the vector, and the reduction of the overall inoculum by the removal of diseased trees or branches, enabled citrus producers to continue to produce citrus for export in areas where HLB once was endemic. However, due to this, combined with an extremely dry decade that was unfavourable for psylla, producers are under the impression that
HLB in South Africa is something of the past. This unfortunately is not so. HLB remains one of the factors limiting citrus production in certain areas of South Africa. Only if the practical strategies which were developed to enable growers to live with the disease are followed, will citrus be produced successfully in such areas.

Areas such as the Eastern Cape are almost free of HLB in spite of approximately 80 000 trees being moved from HLB-infested areas to this area before the movement of trees was prohibited. The reason for this is unclear. On the other hand, areas such as the Citrusdal and Vaalharts are safe because of the environmental conditions being too harsh for the vector to survive.

The time which was gained by researchers such as Buitendag through practical means of controlling the vector is limited. More effective measures to control HLB need to be found. These include resistant varieties and cross protection agents. A new co-ordinated approach to control the disease must be adopted involving relevant Universities, citrus research organisations and producers. Cross pollination of ideas between organisations such as Citrus Research International (South Africa), Fundecitrus (Brazil) and INRA (France) is essential if we intend solving problems such as HLB, CVC and the many other citrus diseases which are limiting the production of citrus all over the world. Researchers, e.g., Bové, Buitendag and Van Vuuren are reaching retirement age and the need for new researchers to be trained by Barry Manicom (ITSC), Lize Korsten (UP) and Monique Garnier (INRA) is essential. If new researchers are not trained we shall be unable to maintain HLB-free orchards in the long term.

Acknowledgement

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Literature Cited