

Reduction of Huanglongbing Due to the ‘Appropriate Techniques Expansion for the Cultivation of King Mandarin’ Project in Southern Vietnam

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Abstract

Our project was the expansion of appropriate cultivation techniques of King mandarin (*Citrus nobilis* Loureiro) to growers who have average-sized farms of 1,000 to 10,000 m² in southern Vietnam. In this region, most citrus trees are infected by huanglongbing (HLB) within two years of planting. The HLB management techniques implemented consisted principally of Nippa palm (*Nypa fruticans*) or Banana (*Musa* spp.) windbreaks on all sides of the orchards, planting of guavas (*Psidium guajava*) one to two months prior to planting disease-free King mandarin trees, and insecticide applications. Insecticide applications were scheduled as follows: 1) soil-drenching with neonicotinoid around King mandarin trees 10 days before planting and every two months for one year thereafter; and 2) leaf-spraying every month from the second year on. In seven of the eleven orchards included in the project, 0.3 to 2.6% of King mandarin trees were infected by HLB in the first year and 3.0 to 21.9% in the second year. However, the infection rate in the first year reached at 21.1 to 39.3% in four orchards where the insecticide was not correctly applied. The results indicate that the appropriate use of neonicotinoid curtailed the infection of King mandarin trees by HLB. The success of our project thus depends on how precisely the techniques are transferred and incorporated by the growers.

Keywords: HLB infection, HLB free tree, neonicotinoid, technique transfer, citrus grower, *Citrus nobilis*

INTRODUCTION

Huanglongbing (HLB), is one of the most serious diseases of citrus (Halbert and Manjunath, 2004), being widely spread in South-East Asian countries (Bové et al., 1996). The disease is a major limitation on the citrus industry in Vietnam (Gatineau et al., 2004, Hoa et al., 2004). The Southern Horticultural Research Institute (SOFRI) and Japan International Research Center for Agricultural Sciences (JIRCAS) established a new management of HLB for King mandarin (*Citrus nobilis* Loureiro) in Southern Vietnam (Chau et al., 2011). The management mainly consisted of: 1) planting citrus young trees when the population density of the vector insect, *Diaphorina citri* Kuwayama, is low (Tuan et al., 2011), and 2) controlling the vector by application of systemic insecticides both ten to seven days before planting and once every two months after the planting for two years (Ichinose et al., 2011b). The management also includes an option of interplanting guavas, *Psidium guajava*, between citrus trees (Ichinose et al., 2012). These techniques are included in a new integrated pest management (IPM) protocol for HLB in southern Vietnam by SOFRI and JIRCAS (Ichinose et al., 2011a; Ohto et al., 2011). Following the establishment of the above IPM, the Japan International Cooperation Agency (JICA) has performed a project transferring appropriate cultivation techniques of King mandarin to citrus farmers in five provinces of Southern Vietnam since October 2009.

In this paper, we report the efficacy of the new protocol for HLB control, and attempt to identify important factors for successful citrus cultivation in the region.

MATERIALS AND METHODS

Farm Preparation before Tree Plantation

Eleven farms, hereafter referred to as A to K, were selected as JICA project farms in Southern Vietnam; two are in Tam Binh, Vinh Long province (farms A and B); three in Mo Cay Bac, Ben Tre province (C, D and E); two in Cau Ke, Tra Vinh province (F and G); two in Ke Sach, Soc Trang province (H and I); and two in Cai Be, Tien Giang province (J and K) (Table 1). All farms had cultivated King mandarins, Da Xanh pomelos (*C. maxima* Burm.), mangoes (*Mangifera indica* L.), bananas (*Musa* spp.), mangosteens (*Garcinia mangostana* L.), coconut palms (*Cocos nucifera* L.) or interplanted any of these crops between others until 2009. These farms had planting ridges of 8 to 9 m in width, which were divided by channels 2 m wide. On each ridge, two lines were set at a 4 to 5 m distance, and planting mounds were made every 4 m on the lines. All planted trees, including citrus, planted contiguously were removed prior to the project.

Table 1. Farming locations, area, and the number of planted King mandarin trees and guava trees on JICA project farms.

Farm	Location (Province)	Farm area (ha)	Number of planted King mandarin trees	Number of planted guava trees
A	Vinh Long	1.0	370	629
B	Vinh Long	1.0	371	545
C	Ben Tre	0.5	197	396
D	Ben Tre	0.7	225	330
E	Ben Tre	0.6	140	306
F	Tra Vinh	0.8	228	570
G	Tra Vinh	0.6	252	476
H	Soc Trang	0.6	315	484
I	Soc Trang	1.0	234	381
J	Tien Giang	0.8	168	486 ^z
K	Tien GIang	1.2	389	760

^zGuava trees had been planted three years prior to planting King mandarin.

King mandarin trees and guava trees were produced at SOFRI or at the nursery center of Vinh Long province. All King mandarin trees for planting were propagated by grafting its scion on Volkamer lemon (*C. volkameriana* Pasq.). HLB-infection of the King mandarin trees was examined by DNA polymerase chain reaction (PCR) analyses, which revealed no infected trees. These trees were treated with 0.2 g imidacloprid (Admire 050EC; Bayer CropScience, Leverkusen, Germany) 10 days before the planting by soil-drenching on the soil surface around the trunk. Nippa palms (*Nypa fruticans*) and bananas were transplanted on the margins of each farm.

Tree Planting and the Following Management

Guava trees were planted in both lines at 2.5 m from the site where King mandarin trees had to be planted. Nippa palms and/or bananas were planted on the margins of each farm as windbreaks. Six to 12 months later, King mandarin trees were planted in farms A and B (18 February 2010) and in the other farms (25 November to 26 December 2010). Planted citrus trees were tied to a stick 10 to 20 cm above ground with vinyl rope, and either bent or cut back 30 cm above the graft. The trees were treated with 0.2 g imidacloprid once every two months after the planting for one year, and twice a month with organophosphate by leaf spraying thereafter. Each tree was given 30 g synthetic fertilizer (20N-20P-16K+TE) once a month for one year, and 90 g after the second year. They were also provided with 10 kg organic fertilizer, 0.5 kg phosphate and 0.3 kg

magnesium-lime every six months. Pruning and training of branches were performed when needed.

Training Course and Extension Works

Training on King mandarin cultivation techniques was given to 15 extension officers at SOFRI in May 2010. The training course consisted of the following five sessions: planting trees, pruning and training trees, soil management, pest and disease control techniques, and postharvest. After returning to their work places, the officers visited project farms in their province one to three times a month and provided techniques that growers should follow.

Inspection of HLB Infection

HLB infection was examined on five mature leaf samples of all King mandarin trees in the eleven farms every six months. When HLB-suspected leaves were found on a tree, these leaves were selected for analysis. For DNA extraction, the midribs of the leaves sampled from each tree were homogenized in 2 ml of CTAB solution (200 mM Tris-HCl, pH 8.3, 100 mM EDTA, 1% CTAB and 1.4 M NaCl) and the homogenate was transferred to a 1.5 ml microtube. After incubating at 65°C for 15 min, the homogenate was centrifuged at 14,000 rpm and 4°C for 1 min, and 0.6 ml of the supernatant were vortexed with 0.6 ml of chloroform-isoamyl alcohol (24:1) and centrifuged at 14,000 rpm and 4°C for 10 min. After decanting the supernatant, it was gently mixed with 0.6 ml isopropanol, and then centrifuged at 14,000 rpm and 4°C for 10 min. The DNA pellet was then washed with 70% ethanol, dried, and re-suspended in 0.1 ml of distilled water.

Two primers of 16s rDNA (Jagoueix et al., 1994) were used for the detection of the HLB pathogen in this study: OI1 (5'GCG CGT ATG CAA TAC GAG CGG CA-3') and OI2c (5'GCC TCG CGA CTT CGC AAC CCA T-3'). The PCR reaction was performed using a peqSTAR thermal cycler (CLEMENS GmbH, Waldbüttelbrunn, Germany) in an 11- μ l reaction mixture containing 1 μ l of DNA template, 1 μ M of each primer and one unit of puReTaq Ready-To-Go PCR Beads (GE Healthcare, UK). The thermocycling conditions included an initial hold at 95°C for 3 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 1 min. The amplified DNA products were electrophoresed in a 1.2% agarose gel in TAE buffer. The gel was stained with 0.5 μ g/ml ethidium bromide and visualized under ultraviolet light of 254 nm.

RESULTS AND DISCUSSION

No HLB infection was detected in farms A and B in six months after planting (Table 2). The first infection occurred in 12 months in both farms, and the proportion of infected trees increased finally to 21.9 and 3.0% in two years, respectively. The infection rates in farms C, D and E were 1.5, 0.4 and 0.0% at 12 months, respectively. Disease rate increased to 4.6, 3.1 and 0.7%, respectively, in 18 months. Infected trees in farms F and G were 3.9 and 6.7% in six months, that rose to 21.1 and 23.0%, respectively, in 10 months. In farms H and I, the infection rate was 0.3 and 2.6% in 12 months, and 6.8 and 3.0%, respectively, in 18 months. Finally, in farm J HLB infection was not detected in the first six months, but it reached 39.3% in 12 months. Such a sudden increase of HLB infection, 24.4 % in the same period, was also observed in farm K.

Table 2. The change in HLB infection rates (% of infected trees) on eleven farms in Southern Vietnam after planting King mandarin trees.

Farm	Infection rate (%)						
	Months after the planting						
	0	6	10	12	18	20	24
A ^y	0.0	0.0	—	0.3	—	13.5	21.9
B ^y	0.0	0.0	—	0.3	—	1.1	3.0
C ^x	0.0	0.5	—	1.5	4.6	—	—
D ^x	0.0	0.0	—	0.4	3.1	—	—
E ^x	0.0	0.0	—	0.0	0.7	—	—
F ^z	0.0	3.9	21.1	—	—	—	—
G ^z	0.0	6.7	23.0	—	—	—	—
H ^x	0.0	0.3	—	0.3	6.7	—	—
I ^x	0.0	0.0	—	2.6	3.0	—	—
J ^x	0.0	0.0	—	39.3	—	—	—
K ^x	0.0	5.4	—	24.4	—	—	—

^zFarms where King mandarin cultivation was canceled after 10 months.

^yKing mandarin trees were planted in February 2010.

^xKing mandarin trees were planted in November to December 2010.

— Samples not collected.

Ichinose et al. (2011b) treated King mandarin trees with neonicotinoid 10 days before planting and planted them in November when the HLB-vector, *D. citri*, density was low. The same insecticides were applied once every two months subsequently. They reported that the HLB infection could be kept at 4.6% or less for one and a half years after planting. Our results also indicated that appropriate use of neonicotinoid curtailed the HLB infection of King mandarin trees. However, farms F, G, J and K showed severe HLB infections. The unexpectedly high infection rate can be attributed to misuse of the insecticides and inadequate treatment of preexisting infected trees. In March 2012, the growers of these farms were enquired on how they had applied insecticides to their mandarin trees and all of them had diluted the insecticide incorrectly or applied it at intervals different from those suggested. Infected trees were left in or near the J and K farms. If the growers had followed the suggestions from officers who received the training courses, these high HLB infection rates would have been avoided. Sekino et al. (2011) reported that some growers achieved high fruit production and a sufficient income by following the suggestions for HLB management in Southern Vietnam. They attributed this success to the establishment of the advisory system for improvement of citrus farming.

The results of our project support the efficacy of the IPM program developed by SOFRI and JIRCAS for prevention of HLB infection, and they indicate that the success of King mandarin cultivation in Southern Vietnam depends on how precisely techniques are transferred to, extended to, and followed by the growers.

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