Incidence of Huanglongbing and Citrus Rehabilitation in North Bali, Indonesia

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ABSTRACT. For many years, Tejakula mandarin, a popular local variety well adapted to the coastal region, was grown in the coastal plains of North Bali. Although Huanglongbing (HLB) was known to be present in Bali, it was thought to have been eradicated during programs in the early 1980s. From 1991 to 1993, up to 1 million Tejakula mandarin trees were planted in this region. Two citrus surveys for HLB were conducted in April 1996 and 1997. Based on symptomatology and the detection of the HLB liberibacter by PCR in leaf samples from over 200 trees, HLB was determined to be present throughout the coastal zones surveyed. Almost 100% of the Tejakula mandarin trees were affected and showed severe stunting. The causal agent was found to be "*Candidatus* Liberibacter asiaticus". Abundant populations of *Diaphorina citri*, the psyllid vector of HLB, were present and in certain orchards up to 15% of insects were HLB positive as shown by DNA hybridization of *D. citri* crush-blots. One of the major findings of our survey relates to the occurrence of HLB as a function of altitude. HLB was commonly present below an altitude of 650 m, however, HLB was rarely seen above 1,000 m. This distribution of HLB probably reflects that of *D. citri*.

Huanglongbing (HLB), known in Indonesia as Citrus Vein Phloem Degeneration, was first reported in 1964 (6). Severe epidemics of the disease occurred in the 1960s, especially in Java and Sumatra where at least 3 million trees were destroyed (5). In 1970 when the disease agent was shown to be a bacterium (2), a large, nation-wide program to control HLB by injecting tetracycline into trees was started, but met with total failure. From 1978 to 1988, the average citrus fruit production was as low as 7.3 tons/ha. A citrus rehabilitation program based on the eradication of infected trees and the production and use of disease-free budwood by shoot-tip grafting was initiated in the mid 1980s (4). Between 1984 and 1987, this resulted in the removal of 3.6 million trees representing about 70% of all citrus in Bali. With the belief that HLB had been eradicated, new trees were planted, especially in the North Bali coastal area (Fig. 1) where the popular Tejakula mandarin variety had been producing well. More than one million Tejakula mandarin on rough lemon rootstock were planted between 1991 and 1993. In August 1993, a groundwater irrigation project supported by the European Union was started as an incentive to farmers to further develop Tejakula mandarin orchards (see Fig. 1 for location of the project area). However, by July 1994, 39% of the newly planted mandarin trees showed HLB symptoms, and by May 1995, the percentage had reached 76%. To deal with this unexpected outbreak of HLB, the irrigation project initiated a new citrus rehabilitation program in 1996. Unfortunately this came to a halt in March 1998, essentially because of the Indonesian economic crisis. In this paper we report on the work carried out on HLB and citrus rehabilitation in North-Bali from April 1996 to August 1998.

MATERIALS AND METHODS

HLB detection. Citrus leaf samples were collected in April 1996 and 1997, and March and August 1998, from orchard trees showing clear or suspect HLB symptoms. Sampling was conducted at 36 sites in the north and north-central regions of Bali (Fig. 1). The Asian citrus psyllid was collected at Penuktukan by mouth aspiration or

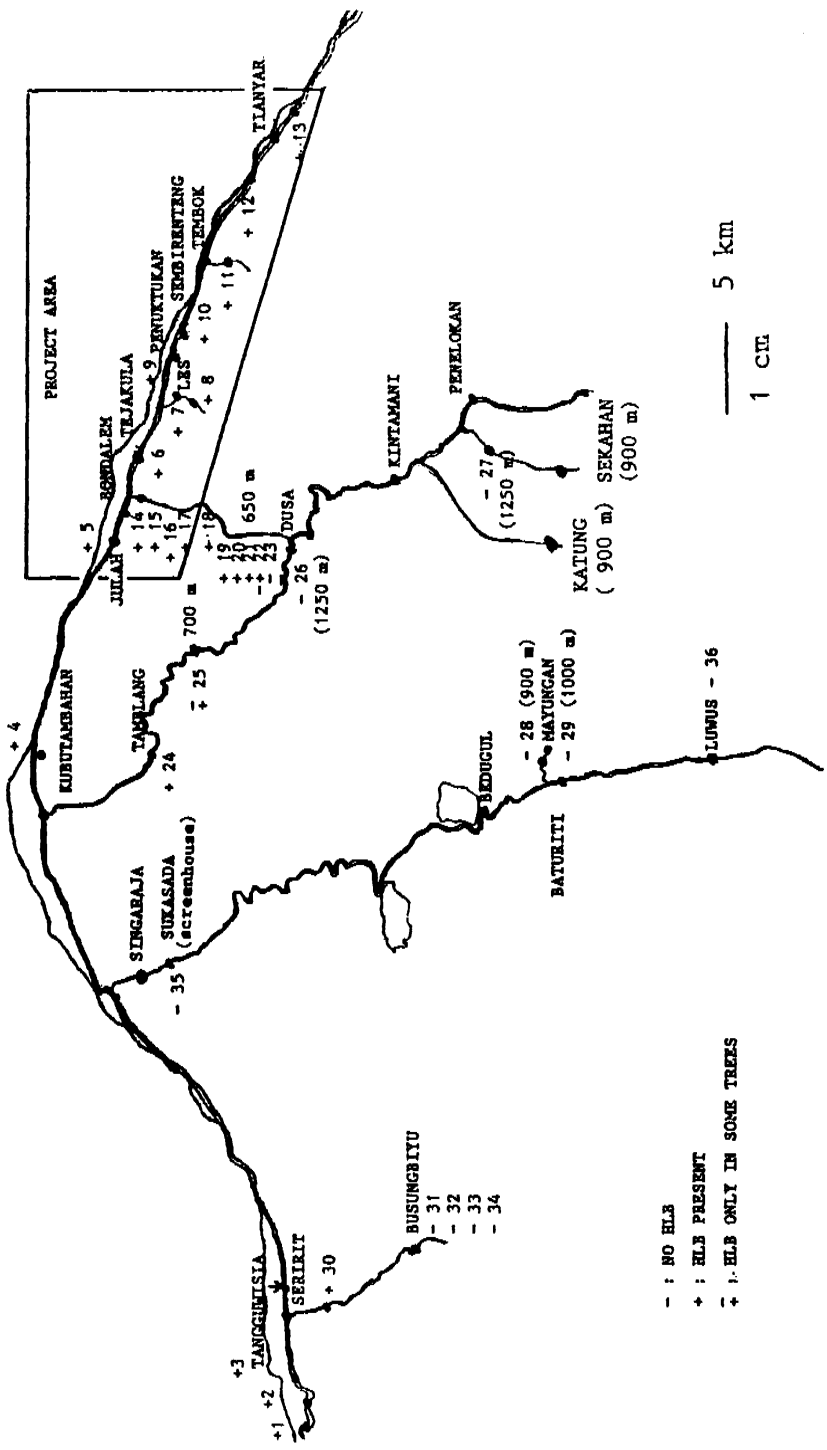

Fig. 1. Map of north and north-central Bali showing the locations of 36 citrus sites surveyed (1 to 36), and incidence of Huanglongbing (HLB) in 1996: - no HLB; +: high % of trees with HLB; ±: HLB in some trees only.

with a D-Vac aspirator. Detection of "*Candidatus* Liberibacter asiaticus", the agent of HLB, was by PCR with primers from the sequence of the 16S ribosomal RNA gene (1) and by DNA hybridization with liberibacter specific probes (3, 7).

PCR and hybridizations for the April 1996 samples were carried out in Bordeaux. PCR for the 1997 and 1998 samples was performed at the Punten extension of the Tlekung Agricultural Research Station (RAJNAT) near Batu, Eastern Java. The Punten PCR laboratory was supported by the Groundwater Irrigation Project and operated by two of the authors (M.E.D. and A.T.) after training in Bordeaux. Both primary production of disease-free citrus budwood at Luwus (Fig. 1) and secondary production of Tejakula mandarin budwood at Sukasada (Fig. 1) were carried out in insect-proof screenhouses from 1994 on. The initial disease-free Tejakula budwood was obtained by shoot-tip grafting from the Tlekung station. Until 1998, Tejakula nursery trees were produced in the open at the government operated Busungbiyu nursery (Fig. 1). In March 1998, the Groundwater Irrigation Project supported the construction of a screenhouse in Amlapura for the production of nursery trees under insect-proof conditions with private sector management.

Trees and psyllid management. The 90,000 Tejakula mandarin trees planted in January 1997 and those planted in 1998 were propagated from screenhouse-produced budwood and raised in the open Busungbiyu nursery. Citrus orchards within 1 km of the nursery were up-rooted in 1995-96 to reduce HLB-liberibacter inoculum.

Diaphornia citri were controlled in the orchards as well as in the Busungbiyu nursery by applications of systemic insecticides (Azodrin = monochrotophos until June 1997 when its use on citrus was banned; Confidor = imidacloprid thereafter) every 2 weeks, and of a contact insecticide (Thiodan = endosulfan until June 1997; perfkthion = dimethoate, after June 1997) at flushing. Until March 1998, vector control was supervised and monitored by the irrigation project; thereafter the private sector took over.

RESULTS AND DISCUSSION

Distribution and incidence of HLB in north Bali in 1996 before citrus eradication. Figure 1 indicates the major sites surveyed in North and Central Bali. The Tejakula mandarin trees examined during the 1996 survey were planted in 1991 to 1993, following the eradication campaign of the 1980s. Surprisingly, almost all the Tejakula mandarin trees along the North Bali coast (Fig. 1) were uniformly stunted with sparse foliage of small, yellow leaves. Many trees showed leaf-mottle which, worldwide, is the most characteristic symptom of HLB. Tejakula expresses good symptoms of leaf mottle, even though mandarin trees generally show less leaf mottle than sweet orange trees.

The small yellow leaves as well as the mottled leaves always tested positive by PCR (Fig. 2) and/or DNA hybridization, demonstrating that the symptoms of trees were due to HLB and not adverse abiotic causes. Three fruit symptoms were also frequently observed on Tejakula mandarins: small size, color inversion, and seed abortion.

On the basis of symptomatology and extensive PCR tests (Table 1, Fig. 2), it was clear that, in 1996, HLB was present throughout the coastal area surveyed which included Tegallingah (Fig. 1, site 1) in the east, to Tianyar (Fig. 1, site 13) in the west. The percentage of affected trees was close to 100%, in particular in the irrigation project area (Fig. 1, sites 5 to 17). The trees,

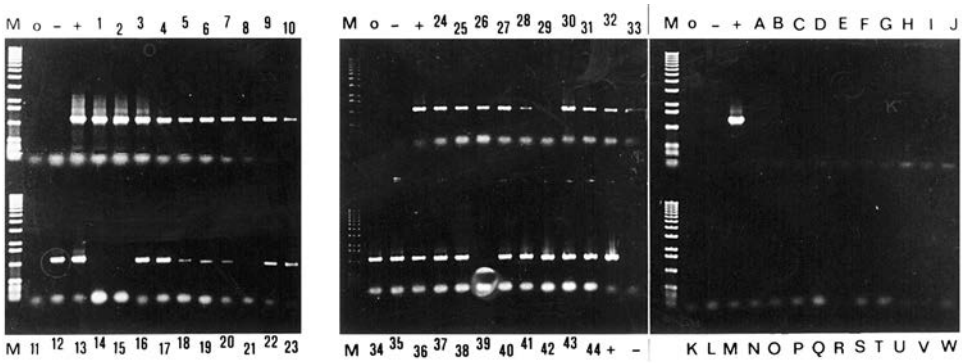

Fig. 2. Agarose gel electrophoresis of DNA amplified by PCR from asymptomatic leaves (7, 11, 14, 15, 21, 29, 39), mottled leaves (1 to 6, 8 to 10, 12, 13, 16 to 20, 22 to 28, 31, 32, 34, 40 to 44) or small yellow leaves (30, 33, 35 to 38) from orchard trees. Sample 7 was from an asymptomatic shoot on a Huanglongbing-affected tree. Samples A to W: Busungbiyu nursery trees; samples were composed of 15 leaf midribs, each leaf from a different plant; - and +: DNA, respectively from healthy and "*Candidatus* Liberibacter"-infected control trees; 0: H_20. The lower band on certain tracks represents primer DNA; upper band: liberibacter specific 1,160 bp amplified DNA; M: size markers. Numbers are the sample numbers, not the site numbers of Fig. 1.

which were less than 5-yr-old, showed severe symptoms of HLB (stunting of tree, small, yellow leaves, low fruit production). The health status of the trees at the time of planting was not known, and some could have been contaminated from the use of infected buds. However, it seems more likely that the high percentage of infection witnessed in 1996 was due to HLB transmission by *D. citri*. Indeed, very high numbers of psyllids could be seen on most of the trees. Autoradiographs of *D. citri*-crush-blots with "*Candidatus* L. asiaticus"-specific probe, In 2.6, showed 6% (36 of 514 insects) of the *D. citri* population to be positive for the HLB pathogen. In one orchard, the percentage was as high as 15% (Fig. 3). High numbers of psyllids were also collected on small fruited acid lime trees.

TABLE 1
PCR-DETECTION OF "*CANDIDATUS* LIBERIBACTER ASIATICUS" IN HUANGLONGBING-SYMPTOMATIC OR SUSPECT TREES FROM NORTH AND NORTH-CENTRAL BALI

Year, Month	Cultivar	No. symptomatic or suspicious trees analyzed	PCR results	
			+	-
1996, April	Tejakula mandarin	42	42	0
"	*Citrus aurantifolia*	3	2	1
"	Rangpur lime	1	1	0
"	Sweet orange	1	1	0
"	*Citrus amblycarpa*	1	1	0
1997, April	Tejakula mandarin	6	6	0
"	Lemon	1	1	0
"	*Citrus amblycarpa*	1	1	0
1998, March	Tejakula mandarin[z]	100	42[y]	58
1998, August	Tejakula mandarin[z]	8	4	4
"	Siam mandarin	14	13	1

[z]Trees planted in January 1997.
[y]0.03% reinfection.

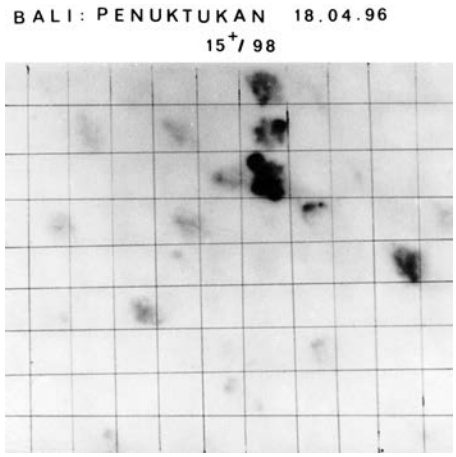

Fig. 3. Autoradiography of crush-blot of *Diaphorina citri* psyllids. Insects were collected at Penuktukan on April 18, 1996, and 98 of them were individually crushed on a nylon membrane; 15 blots gave hybridization signals of which four were strong.

The north Bali coast including the project area is bordered to the south by steep slopes of two volcanoes: Mount Batur (1,717 m) and Mount Agung (3,142 m). The survey was also conducted along the road which climbs over a distance of 16 km from Bondalem on the coast at an altitude of 10 m to Dusa at 1,250 m. As shown by symptomatology and PCR, HLB severely affected many Tejakula mandarin trees up to an altitude of about 500 m (Table 2). At 650 m (Fig. 1, site 22), only one stunted tree with small, yellow leaves as well as leaf mottle could be seen among many green symptomless trees. Leaves from this tree gave a strong positive PCR reaction (Fig. 2, sample 43). At the altitude of 850 m and at higher altitudes, no HLB affected trees could be found. At these altitudes, Siam is the major mandarin variety. The absence of HLB would be explained if Siam mandarin was tolerant to HLB. The 1998 survey showed this not to be the case.

Similar observations were made along the road from Kubutambahan on the coast to Penelokan at 1300 m, or from Singarajah, also on the coast, to Bedugul (1,250 m), Mayungan (1,000 m) and Luwus (450 m) (Fig. 1).

In summary, in North Bali, in April 1996, HLB was present at a very high incidence (close to 100% infected trees) all along the coast and up to altitudes of about 500 m. The incidence declined sharply around 500 to 700 m (Table 2).

HLB in 1997 and 1998 (after citrus eradication). The high incidence of HLB, especially in the irrigation project area, found in the 1996 survey indicated that a prerequisite for citrus rehabilitation was citrus eradication, not only within the project area, but also in buffer zones along the coast east and west of the project area, as well as south up to an elevation of approximately 500 m. Therefore, from late 1996 to early 1997, the first phase of a new citrus eradication project took place in the district of Les and Tejakula (300 ha) and Tianyar-West (200 ha) (Fig. 1). About 90,000 Tejakula mandarin trees on rough lemon were planted in January 1997 in the eradicated zones. These trees were prop-

TABLE 2
NORTH BALI: DISTRIBUTION OF HUANGLONGBING IN 1996 AND 1998: BEFORE AND AFTER CITRUS ERADICATION IN COASTAL AREAS

		1996 Pre-eradication		1998 Post-eradication	
Area & elevation	Cultivar	Age (y)	Incidence	Age (y)	Incidence
Coastal areas (10 m)	Tejakula	4 to 5	100%	Planted Jan. 97	0.03%
10 m to 500 m	Tejakula	4 to 5	High	Planted Jan. 97	Very low
500 m to 700 m	Tejakula, Siam	—	Low	—	50%
700 m to 1,300 m	Siam	—	0 to very low	—	More than in 1996

agated from healthy buds and grown in the Busungbiyu open-air nursery with insecticide sprays every two weeks. PCR revealed no liberibacter infections of the tested nursery trees (Fig. 2, samples A to W). A second eradication campaign was conducted from October to December 1997 covering more than 1,500 ha in the districts between Tejakula and Julah in the Western limit of the project area, and at Tianyar-East, at the Eastern limit. The 1998 survey showed that eradication of citrus was very efficient as no residual orchard or backyard trees could be found.

While the April 1997 survey essentially confirmed observations made from the 1996 surveys in areas where citrus eradication had not occurred, the August 1998 survey brought additional information (Table 2). For instance, in 1996 to 1997, while the HLB incidence in the areas located between altitude 10 m and 500 m was high, it was very low in 1998 as the old symptomatic trees had been removed and the young replanted trees were HLB-free (Table 2). However, in 1996 to 1997, little HLB occurred between 500 and 700 m, in August 1998, 50% of the trees were now affected as shown by symptomatology and PCR. Also, between 700 and 1,300 m, many more trees showed HLB symptoms in 1998 than in the 1996-97 survey. Furthermore, these trees were of the Siam variety (Tables 1 and 2).

The 1998 survey showed an additional citrus zone which includes the Katung and Sekahan areas (Fig. 1) to be affected by HLB. This is the major Siam mandarin production zone. The orchards located between altitudes 700 m to 1,000 m had a high HLB incidence while above 1,000 m, the incidence was still low. Below 700 m, rice is the predominant crop.

The 90,000 or so Tejakula mandarin trees planted in January 1997 within the project area where eradication had been carried out, were surveyed in March and August 1998 for reinfection by the HLB agent. Leaf samples for PCR were taken on symptomatic and/or suspicious trees. In March, only 100 such trees were spotted of which 42 were PCR positives, and as few as eight in August with only four positives (Table 1). Thus, as little as 0.03% trees became reinfected with the HLB agent between January 1997 and August 1998. The infected trees were subsequently pulled out.

These figures do not include 10 trees showing HLB symptoms in August 1998 in a small orchard at Tianyar East; 4 mo earlier only three trees were symptomatic. Here, reinfection is likely due to the fact that the orchard is at the Eastern limit of the project area beyond which no eradication has been carried out. This situation illustrates the need for citrus-free buffer zones around the project area.

CONCLUSION

Citrus rehabilitation was attempted along the North Bali coast in an area where the incidence of HLB was very high. The rehabilitation program involved (i) total citrus eradication in all orchards and backyards; (ii) planting of 90,000 Tejakula mandarin trees propagated from HLB-free buds in January 1997; and (iii) control of *D. citri* by insecticide treatment of the planted trees every 2 weeks. Because replanting started before eradication was totally achieved, *D. citri* was controlled by frequent insecticide treatments. Under these conditions, less than 0.03% of the trees showed HLB symptoms in August 1998. This suggested that the rehabilitation program would probably have met with success had the program continued beyond March 1998.

In north Bali, from 1996-97 to 1998, there was an increase in HLB in Tejakula and Siam mandarin orchards above 500 m and up to

1,300 m. Whether this increase is the result of citrus eradication below 500 m or represents natural spread of the disease, cannot be assessed. A similar increase in HLB has occurred in the Sekahan/ Katung area of north-central Bali where the HLB incidence has become high between 700 and 1,000 m, while it is still low above 1,000 m. Here, natural spread of the disease is involved as no citrus eradication has been carried out in or close to this area.

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