

## SURVEYS AND CERTIFICATION

# Occurrence of Graft-Transmissible Diseases of Citrus in the Highlands of Northern Luzon, Philippines

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**ABSTRACT.** Observations from continuous orchard monitoring and indexing of the graft-transmissible diseases of citrus in the highlands of Northern Philippines are reported. Citrus tristeza virus (CTV) and huanglongbing (HLB) (greening) were found to be the most prevalent diseases affecting almost all citrus cultivars surveyed. CTV symptoms observed included stem pitting, stunting, and small fruit production on all sweet orange cultivars and in a local mandarin called "Gayunan". HLB was fairly common and was causing a debilitating effect on sweet orange and mandarin cultivars. The HLB vector, *Diaphorina citri*, was found in low numbers in a few areas but both the pathogen and vector was observed becoming established even at higher elevations. Co-infection with both diseases was common and this resulted in a more rapid tree decline. Other diseases were found and are reported here for the first time. These include woody gall/vein enation and a budunion disorder of sweet orange on rough lemon rootstock.

Citrus is becoming an increasingly important fruit crop in the highlands of Northern Luzon, Philippines, known as the Cordillera Region. Sweet orange and mandarin plantings once flourished in this region, particularly in Benguet and the Mountain provinces. A local mandarin cultivar called "Gayunan," popular in the local market due to its excellent flavor, and is especially grown in the upper Kalinga province. By 1987, however, these earlier plantings have declined severely and were practically eliminated (C. C. Consolacion, unpublished data). A survey conducted by Roettger et al. (15), showed the widespread occurrence of citrus tristeza virus (CTV) but there was no substantial report on other diseases.

The survey reported here was conducted in support to the revival of the citrus industry as part of the activities undertaken by the Philippine-German Fruit Tree Project. The overall objective of this project is the rehabilitation and expansion of the fruit industry as a whole in the highlands of Northern Luzon.

## MATERIALS AND METHODS

Regular field monitoring and inspection of established citrus orchards and backyard trees was conducted since 1986 in various areas covered by the Philippine-German Fruit Tree Project. To confirm field observations, trees suspected of having virus diseases were indexed. Indexing for CTV was done by ELISA using the double-antibody sandwich ELISA method with polyclonal antibodies purchased from Germany (2). Indexing for the other diseases for conducted by biological indexing.

Biological indexing was carried out at Baguio National Crop Research and Development Center where relatively cool temperatures (avg min. = 14°C; max. = 22°C) prevail throughout the year. The standard procedure of graft inoculation to a range of indicator plants was followed (9, 11, 18). Generally, four to six seedlings were inoculated with three stem pieces per sample. Inoculated plants were kept in an insect-proof greenhouse and regularly

observed for symptom development. A compartment covered with transparent plastic was provided for growing plants and indexing diseases requiring warmer temperatures.

In addition, observations on CTV stem pitting were made on eight twigs (10-15 cm) collected from different sides of the tree, the bark removed, and the degree of stem pitting scored (13).

## RESULTS AND DISCUSSIONS

The different virus and virus-like diseases of citrus which have been previously recorded in the Philippines are CTV, HLB, exocortis, psoriasis, xyloporosis and tatter leaf (1, 6, 7, 15, 19). The present survey in the highlands showed that only two of these, CTV and HLB, were present. In addition, two other diseases, woody gall/vein enation and sweet orange on rough lemon incompatibility, not previously reported, were present.

**CTV.** CTV and its principal aphid vector, *Toxoptera citricida* (Kirkaldy), was common and widespread in the highlands. ELISA tests of tissue samples from representative orchards originally planted with virus-free materials in the different provinces showed an infection rate of 3 to 81% in just 5 yr after planting (Table 1). Low infection was found in orchards planted in relatively isolated areas and in areas where citrus were planted for the first time. The most important and obvious symptom of CTV was stem pitting especially on sweet orange, grapefruit and the local "Gayunan" mandarin. Table 2 shows the stem pitting assessment done on a sweet orange collection in Baguio City. Pitting occurs on the stem, branches and twigs. Twigs of severely pitted trees readily break at the joint when bent and the bark is somewhat thickened and cheesy (9, 11, 12, 13, 16, 17, 20). Other symptoms observed include stunting, poor

TABLE 1  
INCIDENCE OF CTV IN SAMPLE ORCHARDS TESTED BY ELISA FIVE YEARS AFTER PLANTING

Location	No. trees tested	No. trees infected	Infection (%)
<b>BENGUET PROVINCE</b>			
Baguio	235	154	65.5
Caliking, Tublay	131	87	66.4
Nalseb, Tublay	40	14	35.0
Bad-ayan, Buguias	93	3	3.4
Lengaoan, Buguias	151	7	4.6
Buguias Central	78	6	7.7
Pito, Bokod	48	8	16.7
Bolinsak, Kapangan	59	41	69.5
Sagpat, Kibungan	99	34	34.3
Balili, Mankayan	49	40	81.6
Taneg, Mankayan	103	27	27.0
Acmet, Mankayan	49	33	67.3
<b>MT. PROVINCE</b>			
Balangig, Sagada	105	81	77.1
Suyo, Sagada	156	115	73.7
Kin-iway, Besao	88	4	4.5
Macassab, Barlig	49	3	6.1
<b>IFUGAO PROVINCE</b>			
Banaue	278	70	25.1

TABLE 2  
STEM PITTING ASSESSMENT IN SWEET ORANGE COLLECTIONS IN BAGUIO CITY (1993)

Cultivars	Avg stem pitting score <sup>a</sup>
Trovita	2.56
Valencia Late	2.41
Washington Navel	2.29
Gillete Navel	2.25
Atwood Navel	2.45
Hamlin	1.52

<sup>a</sup>Stem pitting score: 0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe.

foliar growth with small, chlorotic leaves. Biological tests done on a number of samples induced varied degrees of severity in Mexican lime, sweet orange and sour orange indicators. Symptoms on Mexican lime included stunting, vein clearing, vein corking, leaf cupping, and thickened and cheesy bark. In Madam vinous sweet orange, symptoms were stunting and stem pitting. Some isolates induced seedling yellows reaction on sour orange indicator seedlings.

Results clearly showed the presence of severe CTV strains which can severely affect susceptible cultivars. This is a serious threat to the highland citrus since growers are reluctant to remove infected trees as these are not killed, unless co-infected with HLB, and continue to maintain the trees regardless of poor yields and small-sized fruits. Co-infection by CTV and HLB were observed to have a synergistic effect in debilitating susceptible citrus cultivars.

**HLB.** The symptoms of HLB as observed in the highlands conform to what has been previously reported and observed by various workers in other citrus growing areas elsewhere (1, 6, 8, 9, 20). Typical symptoms observed included sectoral yellowing and mottling of leaves. Often, severe defoliation occurs and multiple weak and yellow shoots develop. Fruits are small and lopsided with most of the seeds being aborted. Fruits of some cultivars exhibited inverted coloration. Sweet orange

and mandarin cultivars were the most susceptible while limes were more tolerant. Generally, it was observed that infected trees decline in 2 to 3 yr after initial symptoms became obvious.

Results of visual surveys for HLB in the different provinces are shown in Table 3. Infected trees were mostly found in Benguet Province, most probably due to its proximity to the lowland areas where the disease is most prevalent. It is also the major entry point of plant materials from contaminated nurseries in the lowlands. Biological tests using Ponkan mandarin and Madam vinous sweet orange as indicator plants confirmed field observations of the presence of HLB. Symptoms on the indicator plants were observed 4 to 6 mo after graft inoculation.

The insect vector, *Diaphorina citri* Kuwayama, was found only in few areas and its population was relatively low. To date, however, the vector continues to appear in new areas and is establishing itself even at higher elevations.

**Woody gall/Vein enation.** Symptoms of woody gall/vein enation were observed in five orchards. These symptoms included large swellings or galls on trunks and branches. The galls were pitted on the cambial surface of the wood and were light green in color (4, 5, 20). Enations were never observed in the field, except on experimentally inoculated rough lemon and Mexican lime seedlings indicator plants.

TABLE 3  
OCCURRENCE OF HUANGLONGBING IN DIFFERENT PROVINCES IN THE CORDILLERA (1993)

Location	No. orchards surveyed	No. trees	No. of symptomatic trees <sup>a</sup>	Infection (%)
Benguet	27	1134	748	66.0
Mt. Province	16	1482	72	4.5
Abra	7	315	17	5.3
Kalinga/Apayao	6	120	18	12.0
Ifugao	10	300	59	19.0

<sup>a</sup>Data based on visual observation of typical symptoms. Representative samples were indexed.

One of the affected fields was a 15-yr-old Meyer lemon orchard planted with marcots having almost 50% of the trees showing advance galling. This seems to be the first report on Meyer lemon. Del Rosario and Alaban (7) also reported vein enation on local mandarin cultivars Ladu and Szinkom in Batangas province, but transmission experiments were not performed to confirm observations. According to the orchard owner, the occurrence of galls did not significantly affect yields.

Other orchards where the disease was found were newly established orchards on rough lemon and volkameriana rootstocks where only few trees show initial gall development.

Budwood samples collected from these orchards were indexed on rough lemon seedlings. All inoculated plants induced vein enation 3 mo after inoculation. Enations were seen on young, fully expanded leaves. Galls developed, however, only on some seedlings one year after inoculation.

In Peru, some workers reported severe galls, stunting and decline in young orchards. Older orchards showed gall development but yields, in general, were not reduced; whereas the trunk girth was somewhat reduced (4).

**Budunion disorder of sweet orange budded on rough lemon.** Several workers have reported disorders on certain scion and rootstock combinations (3, 5, 14). An observed

symptom was a pitted ring in the trunk wood along the budunion. The bark showed a corky collar of eruptive bark encircling the trunk. The affected trees were bushy due to the production of multiple shoots. Leaves were abnormally large, thick and pale green in color; rinds were thick and fruits had less juice and had enlarged peduncles. These symptoms were observed in navel orange budded on rough lemon rootstocks in a citrus collection in Baguio City and in one orchard in Ifugao Province.

Propagations from the infected tree on rough lemon rootstock showed symptoms similar to the observed field symptoms. After 4 mo, all plants showed abnormally large and thick leaves but internal symptoms were produced only after 2 yr.

Recently, Navarro, et. al. (14) showed that it is possible to eliminate a similar disorder through shoot-tip grafting, indicating that the problem is produced by a graft-transmissible agent.

As a result of the survey, a concerted effort of all concerned have resulted in the following recommendations: 1) strengthening of the local quarantine checkpoints at the major entry points to the Cordillera Region by the Department of Agriculture and the Local Government Units to prevent further introduction of contaminated plant materials and the insect vectors from nurseries in the lowland areas; 2) encouraging the establishment of commercial nurser-

ies in the region and the strengthening of the local citrus certification program to ensure the production of quality plant materials which are pathogen-free (to date, 22 nurseries have registered under the program); 3) continuous training program for farmers and local government technicians on orchard management; and 4) continuous research on the control of citrus diseases.

The authors believe that the future of citrus industry in the Cordillera region still holds promise as

long as the above measures are fully implemented by all concerned.

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