VIRUS FREE MATERIALS IN FRUIT TREES

Akira YAMAGUCHI *

There are no fundamental differences between viruses that infect fruit trees and those infecting herbaceous plants. However, because fruit trees are perennial plants, viruses in fruit trees are especially important because; 1) Fruit trees are propagated asexually and once they are infected with virus, the virus usually spreads by propagation, 2) Grafting often results in two or more viruses harbored in the same tree, 3) When a tree has been infected with a virus, it usually takes a long time before the symptoms appear. Sometimes, a symptomless infection occurs and/or a symptomless infection follows shock symptoms, 4) Most commercial fruit trees consist of a rootstock variety, a scion variety, and sometimes an interstock variety. All of which can differ greatly in their sensitivity to virus infection. Therefore, symptoms often depend on the combination of varieties used.

Why virus free materials are important in fruit trees?

Although some fruit tree viruses are transmitted by insects, nematodes, pollen, or seeds, many viruses can be transmitted only by grafting. The only methods by which some of these viruses are known to spread are by the grafting techniques used to propagate commercial trees. For these viruses the absence of natural vectors makes quarantine and certification programs effective methods for controlling spread. Infection can be prevented simply by using virus free varieties for grafting purposes.

Apples Latent apple viruses, chlorotic leafspot virus (CLSV), stem pitting virus (SPV),or stem grooving virus (SGV) are observed throughout all apple growing areas in Japan. Of these, the CLSV-type strain is the most important virus economically. It causes takatsugi-byo (topworking disease) of apple.[8]

This virus was introduced into Japan with Delicious type scions in which it remains latent. In maruba-kaido (*Malus prunifolia* var. *ringo*) rootstock which is widely used in this country as a rootstock of commercial apple cultivar, the CLSV-type strain produces wood pitting and necrosis of the bark. Topworking of the new variety of Delicious type infected with CLSV onto old cultivars on maruba-kaido rootstocks was performed extensively during 1930's. After a few years topworked apple trees began to decline and many ultimately died. This virus is only transmitted by grafting. Distribution of scions for topworking from virus free mother trees is the best and the only method for controlling this disease. It was known that many dwarfed rootstocks are infected with CLSV-type strain. Consequently, now we are very careful not to introduce CLSV-infected materials from foreign countries.

Japanese pears Some cultivars of Japanese pear react with necrotic spots on leaves when infected by pear necrotic spot virus.[8] Symptoms appear from early to middle June, never before, and then only on mature leaves. Leaves of affected trees turn yellow and defoliate during summer. After a few years trees seem to weaken. The most sensitive cultivar is 'Nijisseiki'. one of the best commercial cultivars in Japan. On the other hand, some cultivars experience infection without symptoms. These trees seem to be tolerant to the virus. It is important to know whether a cultivar or a new hybrid clone is tolerant or sensitive (symptoms expressing) before distribution. As this virus is known to be transmitted only by grafting, using virus

* Plant Pathologist, Fruit Tree Research Station, Hiratsuka, Kanagawa 254, Japan.

free scions for topworking or producing nursery trees is the best method to control the disease.

Stone fruits Prunus necrotic ringspot virus (NRSV) and prune dwarf virus (PDV) have been isolated from some symptomless stone fruit trees in Japan.[8] Fortunately, their distribution in Japan appears to be restricted. These viruses can be transmitted by infected pollen and through seeds. Isolation or eradication of infected trees is recommended for control of the disease. Virus free trees must be used as pollen donors for hybridization in breeding.

Citruses Satsuma mandarin covers the greatest acreage among the various kinds of citrus grown in Japan. Satsuma dwarf virus can be transmitted through infected soil as well as by grafting.[7] Elimination of the infected tree and fumigation of the soil are necessary for the control of the disease. Replanting of virus free trees is recommended.

Exocortis viroid has been found only in a restricted area,[7] but has increased in importance because of the use of exocortis-sensitive rootstock, trifoliate orange. The viroid is very stable, resistant to heat, can persist a long time and spread easily by the use of cutting knives. To control exocortis, the use of virus free budwood, sanitary budding, nursery and field practices are necessary.

Grapevines Grapevine fanleaf virus is transmitted by grafting and by nematodes present in the soil. To control the disease, planting of virus free trees and soil fumigation are necessary.

Virus certification of fruit trees in Japan

In 1961, the Japanese government settled on a mother orchard system to fulfill farmers' need for a continuous supply of healthy and true-to-type fruit trees. The legally controlled inspection of virus diseases of citrus and apple by government plant inspectors began. Since 1968, this measure has been extended to stone fruits and grapevines. Mother trees are designated by prefectural government in orchards of farmers or prefectural horticultural experimental station after inspection for known viruses by prefectural researcher. Government plant inspector

Table 1. Indicator plants for indexing viruses of mother trees currently used by Plant Protection Station.

Fruit tree	Diseases	Indicator plants
Citrus	Satsuma dwarf	*Sesanum indicum* (white sesame)
	Hassaku dwarf (CTV-stem pitting str.)	Mexican lime (*Citrus aurantifolia*)
	Exocortis viroid	Etrog citron (*Citrus medica*)
Apple	Apple topworking (CLSV-type str.)	Maruba kaido (*Malus prunifolia* var. *ringo*)
	(SPV)	Mitsuba kaido (*Malus sieboldii*)
Prunus	NRSV and PDV	Shirofugen (*Prunus serrulata*)
		Cucumber (*Cucumis sativus*)
		Squash (*Cucurbita maxima*)
Sweet cherry	GRMV	Kwanzan (*Prunus serrulata*)
Grapevine	GFV	*Chenopodium amaranticolor*
		C. quinoa
		Gomphrena globosa

CTV: Citrus tristeza virus; CLSV: Apple chlorotic leafspot virus; SPV: Stem pitting virus; NRSV: Prunus necrotic ringspot virus; PDV: Prune dwarf virus; GRMV: Green ring mottle virus; GFV: Grapevine fanleaf virus

carefully observes all mother trees annually for the presence of any virus symptoms on leaves or stems. After careful observation, some suspected trees are indexed for viruses using herbaceous or woody indicator plants. Indexing of plants routinely used by the Plant Protection Station is shown in Table 1.

Horticultural associations or some similar organizations ask commercial nurseries to grow nursery trees using scions from the inspected mother trees. Thus farmers can get certified scions for topworking or nursery trees made with scions from the mother trees. Although it seems difficult to estimate with accuracy the exact ratio of the number of certified scions to all scions demanded in our country, designated mother trees of each cultivar of each fruit

Table 2. Number of mother trees and the possible number of nursery trees made from them as compared with the number of nursery trees produced in 1975 in Japan.

Fruit trees	Number of mother trees	Possible number of nursery trees made from scionwood of the mother trees	Number of Nursery trees produced and sold in 1975
Satsuma (*Citrus unshiu*)	45,422	3,529,500	3,130,600
Natsudaidai (*C. natsudaidai*)	5,535	375,000	1,039,600
Navel orange (*C. sinensis*)	16,952	251,100	406,200
Hassaku (*C. hassaku*)	1,674	317,500	877,600
Iyokan (*C. iyo*)	310	16,000	221,100
Apple (*Malus pumila demestica*)	777	469,600	1,136,400
Peach (*Prunus persica vulgaris*)	468	207,200	944,300
Sweet cherry (*Prunus avium*)	15	–	208,700
Grapevine (*Vitis*)	1,451	148,900	886,700
Japanese pear (*Pyrus serotina*)	2,157	245,800	671,900

(Fruit and Flower Division, MAF, 1976)

tree may not be sufficient to meet the demand of farmers. Table 2 shows the number of mother trees, the possible amount of nursery trees made from them and the number of maiden trees produced in 1975. From the Table, it can be easily recognized that the farmer's demand is far beyond the supply of certified trees except for satsuma mandarin. Therefore, it seems desirable that the government select and designate the virus free nuclear mother trees, maintaining and propagating them and distribute virus free materials to farmers as in the IR-2 project in America,[2] the EMLA scheme in England[1] on deciduous fruit trees and the CVI program in California[6] on citrus fruit trees.

How to obtain virus free materials?

Some virus free cultivars can be found by indexing a number of trees for virus content. In the case of apples, it may take over 5 years to detect all known viruses. However, once some cultivars are certified to be free from all known viruses, those cultivars are very valuable and have to be maintained with care to prevent reinfection. Mother tree blocks of these cultivars will be propagated and scions for topworking supplied from them to nurseries.

If all trees of some cultivars or clones of a variety are infected with viruses, it is necessary to produce virus free materials by heat treatment or meristem or tip culture. Sometimes heat treatment is combined with tissue culture.

Heat treatment Heat treatment of virus infected plants has been attempted by exposing whole plants or budded plants to high air temperature or by soaking plant parts in hot water.

Exposure periods and the temperatures depend upon the kinds of tree fruits and the viruses.[3,5] Tsuchiya et al (personal communication) successfully applied thermotherapy for CLSV-type strain-infected Fuji-colored clone and apple rootstock M 9 by heating the plants at 38°C ± 1°C for 25 and 50 days, respectively. Yanase (personal communication) obtained latent virus-free maruba-kaido, indicator of CLSV-type strain, by exposing the plants to 37°C for 3 weeks. This offers researchers clean indicator plants which are necessary for standardizing scientific work. Ohmori et al (personal communication) successfully applied thermotherapy for citrus tristeza virus (CTV)-stem pitting strain which had infected Washington navel budsticks by dipping the plants in 50°C hot water for 1-4 hours after pretreatment at 40°C (daytime)/35°C (night) air temperature for 14 weeks. Yamada (personal communication) tried to free CTV-stem pitting strain from citrus hybrid clone 6781 (satsuma mandarin 'Miyagawa wase' X a clone of trovita orange) which was completely infected in the field after breeding. He obtained a virus free clone by exposing the plants to 40°C (daytime)/30°C (night) for 5 weeks. As CTV is transmitted by aphid (*Toxoptera citricidus*), almost all citrus trees are easily infected in the orchards.

CTV-stem pitting strain greatly damages hassaku (*Citrus hassaku*). This virus can be easily transmitted by aphid. Therefore, planting virus free trees is not of significant value for the control of the disease. In some prefectures, apparently healthy old trees were designated as mother trees as early as 1960 and it was suggested to take scions from these mother trees. Later, it was discovered that these apparently healthy trees contained mild strain of the virus. Now it is thought that the inoculation of virus free trees with mild strain of the virus prior to planting might interfere with a severe strain in the field. Here again, virus free materials are needed.

Tissue culture The use of meristem or shoot tip culture for obtaining virus free materials has been tried successfully with many herbaceous and bulbous plants,[4] but this technique is not yet applicable to fruit trees. Attempts to apply this method to fruit trees have just begun. In the Rutaceae, plants arising from embryogenesis of the nucellus have been shown to be free of most viruses. Unfortunately, such plants show reversion to the juvenile state and the associated undesirable qualities are manifested. Navarro et al. developed a procedure consisting of placing a 0.15 mm long shoot tip excized from an infected tree onto a decapitated rootstock seedling under aseptic conditions, and obtained cultivars free from tristeza virus and exocortis viroid. Kobayashi and Ikeda (personal communication) are now following this technique. Attempts to develop tissue culture of *Malus* (Abbott and Whiteley, 1976) and of *Vitis* (Hirabayashi et al, 1976 and Krul and Worley, 1977) have potential values in mass production of virus free materials.

Conclusion

Horticulturists have faced many troubles such as weakness of trees, incompatibility of grafting, early or late ripening of fruits and so on. Recently, some of these troubles have been shown to be due to virus infection. Breeding of fruit trees usually takes a long time. Sometimes valuable hybrid clones suffer infection with viruses by pollination or insect transmission. Many of these troubles can be avoided with caution and some knowledge of how specific viruses are spread. Replacing all fruit trees with virus free trees may eventually contribute greatly to increased production and good quality fruits.

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Discussion

N. Murata, Japan. Would you explain how you demonstrate the existence of mild strains of virus in satsuma mandarin trees? Is it more desirable to have virus free stocks or stocks harbouring mild strains of virus?

Answer: Mild strains of citrus tristeza stem pitting virus can be indexed using woody indicator, such as Mexican lime (*Citrus Aurantifolia*). The plant reacts to a mild strain of virus with less leaf symptoms and stem pitting lesions than when exposed to a severe strain of virus. It is preferable to use the hassaku plant infected with a mild strain of CTV. The use of virus free plants is meaningless because these are readily infected with viruses transmitted by aphids.

J. T. Rao, India: It is good to have a plant pathologist amongst us breeders. Plant pathologists can take up some of the load from the plant breeder. Heat treatment of sugar cane seed material has resulted in seed material free from two virus diseases, namely ratoon stunting disease and grassy short which cause yield decline in the crop. As an improvement over hot water treatment, is there another steam therapy in which the germination of bud would be better?

Answer: I am happy to hear that you were able to get rid of virus diseases in sugar cane. In general, conditions of heat treatment for getting virus free materials depend on the combination of virus and trees. Sometimes, we face difficulties, such as deleterious influence on tissue growth, etc. Therefore, we must repeat tests until we meet optimal conditions in each combination.

M. Kobayashi, Japan: Why have not you applied yet tissue culture techniques to get virus free fruit trees?

Answer: Indexing a number of trees for virus content is rather economical and convenient. If all trees of some cultivars or clones are infected with viruses, we will try to apply tissue culture methods.

T. Akihama (Comment): We are attempting to apply tissue culture to *Vitis*. According to our experience, this technique can be useful in mass production of virus free fruit trees.

Y. Ohta, Japan: As you explained it, the breeding of virus free plants is quite commendable. However there are drawbacks too. For instance, virus free plants have to face the risk of possible reinfection which may cause more damage than otherwise, as shown in the case of potato. On the other hand, if one gives the crop some immunity against more virulent virus strains, by exposing them to mild or less virulent strains or even by incorporating into them virus particles, one could get better results, as indicated in the case of tomato and citrus.

Answer: I agree with you. Particularly in the case of citrus tristeza virus, planting virus free trees is of no significance for the control of the disease and confering immunity to the crop is far preferable. However, in the case of apple topworking disease and others, in which only graft transmission is known to take place, there is no reinfection and good results can be obtained. The IR-2 project (Virus Certification Program) conducted in the USA or the EMLA scheme in England are representative examples.