Comparison of Hassaku Dwarf and Seedling-Yellows Viruses

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A VIRUS or virus complex inducing a severe stem-pitting reaction in Mexican lime and severe yellows in seedlings of Kawano natsukan (an early variety of natsudaidai) was found in 1967 in field-grown Washington navel orange and satsuma trees in Hiroshima Prefecture. The purpose of this paper is to report the results of experiments designed to clarify the relation between this virus or virus complex (KSYV) and hassaku dwarf virus (HDV).

Materials and Methods

VIRUS SOURCES.—Two main virus sources were used in these experiments: HDV-34, an isolate from a naturally infected hassaku tree with severe symptoms; and KSYV-1, an isolate of Kawano natsukan seedling-yellows virus from a Washington navel orange tree whose twigs were much pitted. Other isolates of the 2 viruses, from other trees, were also used (Table 1).

INOCULATION METHODS.—The viruses were transmitted either by tissue grafts (bud-inoculation) or by means of the aphid *Toxoptera citricidus* Kirk. Graft-inoculated indicator plants were cut back to a single stem 15 cm high to stimulate new growth. The methods of transmission by aphids will be described elsewhere in this paper.

INDICATOR PLANTS.—Potted greenhouse grown plants of various citrus species were used as indicators. They were either healthy seedlings on their own roots or, in the case of Mexican lime, plants propagated as cuttings from healthy parents.

Composite indicator plants grown in pots were also used. Some were formed by topworking healthy Valencia scions onto healthy seedling stocks of sour orange or trifoliate orange. The number of indicator plants in any experiment was 3 to 5 per batch. Virus was introduced into these trees by bud-inoculation. All experiments were carried out in a greenhouse.

Experimental Results

REACTION OF KAWANO NATSUKAN SEEDLINGS.—The conspicuous symptoms produced in Kawano natsukan seedlings by KSYV are severe yellowing of new leaves and severe stunting. A few pits occasionally develop in stems of the new growth.

HDV never causes severe yellowing but does cause severe stem pitting, grooves or furrows on stems, and mild stunting.

REACTION OF OTHER CITRUS CULTIVARS.—The results of inoculating Mexican lime, sour orange, Eureka lemon, and composite trees of Valencia on sour orange or trifo-

liate orange with any 1 of 3 isolates of KSYV or any 1 of 2 isolates of HDV are in Table 1.

The reaction of lime plants to all isolates of the 2 viruses was the same: severe stem pitting and severe stunting. The other indicators reacted differently. Each of the 3 isolates of KSYV caused severe yellowing and stunting in sour orange and Eureka lemon seedlings. The 2 HDV strains failed to produce yellows in sour orange and Eureka lemon, but caused mild stunting and severe pitting in sour orange and no typical reaction in Eureka lemon.

Composite trees of Valencia on sour orange reacted to KSYV isolates by yellowing of the scion leaves and stunting, which is probably the tristeza reaction described by McClean (6). Those infected with the HDV isolates have not shown such reactions. Composite trees of Valencia on trifoliate orange stock showed only occasional vein clearing in the scion leaves when infected with KSYV or HDV.

A few grapefruit seedlings inoculated with KSYV reacted with severe yellowing, stunting, and some vein corking but not with typical stem pitting. Such seedlings reacted to HDV, on the other hand, with vein clearing, severe pitting, and stunting, but not typical yellows.

Fraser in Australia (2, 3) and McClean in South Africa (5, 6) reported similar differences in the behavior of indicators to tristeza virus isolates from different sources. Their isolates from sweet orange induced reactions similar to those induced by KSYV, whereas their isolates from grapefruit or Eureka lemon induced reactions more like those induced by HDV.

TABLE 1. COMPARISON OF THE SYMPTOMS—STEM PITTING (STP), YELLOWING (YLG), STUNTING (STG), AND VEIN CLEARING (VCL)—PRODUCED IN VARIOUS INDICATOR PLANTS BUD-INOCULATED WITH KSYV AND HDV

Virus isolate	Mexican lime cutting		Sour orange seedling			Eureka lemon seedling		Grapefruit seedling		Valencia on sour orange		Valencia on trifoliate orange		
	STP	STG	YLG	STG	STP	YLG	STG	STG	STP	YLG	STG	VCL	YLG	STG
KSYV-1	S[a]	S	S	S	—	S	S	S	—	S	S	M	—	—
KSYV-2	S	S	S	S	—	S	S	S	—	S	S			
KSYV-4	S	S	S	S	—					S	S			
HDV-34	S	S	—	M	S	—	—	S	S	—	—	M	—	—
HDV-841	S	S	—	M	S	—	—			—	M	M	—	—

a. S, severe; M, mild; —, not produced.

NONMULTIPLICATION OF KSYV IN TRIFOLIATE ORANGE.—To determine whether or not KSYV multiplies in trifoliate orange, 1-year-old seedlings were graft-inoculated; 60 days later, buds from the new growth were inserted into the stems of Kawano natsukan and Mexican lime seedlings. Half the inoculated Kawano natsukan seedlings were, 49 days later, reinoculated with a source of HDV. None of the seedlings

inoculated only with buds from the trifoliate orange seedlings developed symptoms. Those doubly inoculated with trifoliate orange buds and with a source of HDV developed vein clearing and stem pitting, a reaction normally induced by HDV alone. The results indicate that KSYV, like HDV, is unable to multiply in the trifoliate orange.

APHID TRANSMISSION TRIALS.—The periods of retention of KSYV and HDV in the aphid vector *T. citricidus* were determined. Twenty-five apterous aphids per plant—5 adults and 20 nymphs—fed for 24 hours on plants infected either with KSYV or HDV were transferred to healthy Kawano natsukan seedlings and allowed to feed for periods of 24, 48, and 72 hours, respectively, before being transferred to fresh healthy seedlings. A high percentage of the healthy Kawano natsukan seedlings that aphids fed on immediately after their transfer from plants diseased with either KSY or HD became infected—11 of 13 for HDV and 12 of 17 for KSYV. The longer the viruliferous aphids fed on healthy plants before being transferred to fresh plants, the lower the percentage of infection in the fresh plants. Apparently the aphids retained KSYV for less than 24 hours while actively feeding, and HDV for less than 48 hours.

HEAT TREATMENT TEST.—Mexican lime seedlings, inoculated with either KSYV or HDV, were kept for 4 weeks in a heated glass chamber at a temperature of 38–40°C. Other inoculated lime seedlings were kept in a greenhouse at a temperature below 35°C.

No visible symptoms developed in the new growth of the heat-treated plants. Neither virus was detected in the new growth of the heat-treated healthy lime plants just after heat treatment. All the heated seedlings developed a severe lime reaction, usually within 6 months, after being transferred to the greenhouse.

CROSS PROTECTION BETWEEN HDV AND KSYV.—Three healthy Kawano natsukan seedlings were exposed to aphids infective for HDV; 6 months later they were bud-inoculated with KSYV. The doubly inoculated seedlings developed vein clearing, stem pitting, and mild stunting, but no yellowing. The results suggest that HDV may have a protective effect against KSYV, at least in Kawano natsukan seedlings.

EFFECT OF KSYV ON HASSAKU SCIONS GRAFTED TO VARIOUS ROOTSTOCKS.—Two sources of hassaku scions were used: one (hm-50), though normal in appearance, carried a mild strain of tristeza virus (10); the other (hs-34) showed visible symptoms of hassaku dwarf. The 2 sources were grafted separately to various rootstocks. Some of the trees propagated from hm-50 were graft-inoculated with isolate 1 of KSYV, others with isolate 34 of HDV, and still others were not treated. Trees grown from hs-34 were not treated, with few exceptions.

Within 6–7 months, the new growth of the hm-50 trees on Kawano natsukan stocks inoculated with KSYV became severely yellowed

and almost ceased growing. Those on natsudaidai and Eureka lemon stocks also yellowed, but the yellowing became milder and more obscure as the new leaves matured. The trees on the other stocks developed no symptoms in their scions.

The scions of all trees inoculated with HDV, irrespective of the stock, developed stem pitting but no yellowing of the new growth; some of them developed grooves or furrows on stems. Their reaction was the same as that of the control scions propagated directly from hs-34.

The reaction of the untreated trees propagated from the 2 scion sources, irrespective of the stock, was the same as their parents: the hm-50 scions grew normally; hs-34 scions developed symptoms of severe stem pitting and grooves or furrows on stems.

Discussion and Conclusions

The yellows reaction caused by KSYV in Kawano natsukan seedlings agrees clearly with the seedling yellows of Eureka lemon and sour orange, first described from Australia (2) and later from other countries (1, 7, 12). *T. citricidus* is common on citrus in Japan, and it is reasonable to assume therefore that KSYV is present in a high percentage of sweet orange and satsuma mandarin trees, as was noted by Miyakawa (8).

HDV, which in Japan is considered to be a severe strain of tristeza virus (9, 10, 11), seems to be closely related to the tristeza virus that causes stem-pitting disease in other countries (2, 3, 4, 6, 7). It seems to be particularly closely related to a severe strain of stem-pitting virus described in Australia—one said to be unable to cause tristeza disease in the sensitive tree of sweet orange on sour orange stock though causing severe stem pitting in grapefruit (3).

Various theories are given to explain the production of seedling yellows and stem pitting by the tristeza virus complex (1, 3, 4, 6, 12). Most agree that within the complex there is an association of at least 2 components or 2 separate viruses, 1 being responsible for the seedling-yellows reaction and another for stem pitting. Some isolates produce both stem pitting and yellows in the respective indicators, lime and sour orange or Eureka lemon, some stem pitting only; but none so far have been capable of inducing yellows without also inducing stem pitting in lime.

In the present work HDV and KSYV were found to resemble each other in producing a severe stem-pitting reaction in the Mexican lime. Neither was able to multiply in trifoliate orange; both failed to multiply at high temperatures in susceptible species. Viruliferous vectors lost their ability to transmit both viruses after feeding on healthy plants for 24–48 hours. HDV seemed to have a protective effect against KSYV in Kawano natsukan seedlings. The relation between the 2 viruses seems to be much the same as that between the stem-pitting and yellows viruses described by McClean

(6), and it is thought that the stem-pitting component and the yellows component are strains of a single virus.

McClean distinguishes a tristeza reaction in addition to seedling yellows and a lime reaction (6). He defined tristeza as the reaction induced in composite trees of sweet orange on sour orange stocks. The symptoms shown in this reaction by sweet orange scions are said to be caused indirectly by the action of the virus on the sour orange tissues just below the union. Not all tristeza virus isolates induce this reaction. In the present work, test plants of Valencia on sour orange showed a tristeza reaction when inoculated with the KSYV isolates, but not with the HDV isolates under greenhouse conditions. There was also apparently a tristeza type of reaction produced in the trees of hassaku (hm-50) topworked on 3 stocks and then inoculated with KSYV. The action was most pronounced in the trees on Kawano natsukan and very mild in the case of the trees on Eureka lemon and natsudaidai. The milder reaction might be due to some protection afforded by the mild strain of tristeza virus initially present in the hassaku scion, hm-50.

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