Heat Tolerance of Preconditioned Citrus Budwood for Virus Inactivation

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SUCCESSFUL thermotherapy of citrus budwood for virus inactivation has been reported for tristeza (2, 5), psorosis (5), exocortis (13), and yellow shoot (8), but some attempts at heat inactivation of citrus viruses have failed (3, 5, 9, 11). Acclimatization or preconditioning of plants before heat treatment increases the heat tolerance of some hosts (1, 4, 7, 10), but its effect on citrus apparently has not been previously reported. This paper reports experiments showing that high temperature preconditioning increases the heat tolerance of citrus and facilitates inactivation of some citrus viruses by heat treatment.

Experiments and Results

EXPERIMENT 1: PRECONDITIONING OF CITRUS BUDWOOD.—Preliminary tests indicated that citrus budwood would tolerate hot water temperatures of 48 and 50°C for 1 hour, and preconditioning might permit prolonging the treatment. Therefore, Pineapple sweet orange seed-

lings were preconditioned for 28 weeks in a greenhouse compartment at 38–42°C maximum and 30–31°C minimum temperatures, in August 1965. Comparative controls were kept in another room at 27–33°C maximum and 20–22°C minimum temperatures. Groups of 6 budsticks, 8 cm long, from plants in each room were immersed in agitated hot water at 48 and 50°C for 2 and 3 hours. Then, 20 buds from each group, including untreated controls, were grafted promptly to 2 rough lemon seedlings. Six weeks later, buds were evaluated for survival and growth. Ninety-three per cent of buds from preconditioned shoots survived heat treatment compared with 33 per cent for nonpreconditioned buds (Table 1). Preconditioned buds treated at 50°C for 2 and 3 hours showed 90 per cent survival compared to 5 per cent for nonpreconditioned buds. Surviving buds from all treatments grew. These results indicated the value of preconditioning for increasing the heat tolerance of citrus.

EXPERIMENT 2: HOT WATER TREATMENTS OF VIRUS-INFECTED CITRUS.— Viruses and hosts were: exocortis in mandarin, concave gum in sweet orange, tristeza in Mexican lime, vein enation in Mexican lime, and stubborn from 3 sweet orange donors. All plants were preconditioned in the warm room for 12 weeks beginning in May 1966, with the exception of a stubborn sweet orange control held at normal greenhouse temperatures for comparison. Temperatures in the preconditioning room were similar to those in experiment 1 except for longer days and occasional maximums of 44°C. Shoot sections 10 cm long from each plant were immersed in agitated hot water

TABLE 1. THE EFFECT OF PRECONDITIONING ON SURVIVAL OF PINEAPPLE SWEET ORANGE BUDWOOD TREATED IN HOT WATER

Budwood source	Hot water treatments		Bud survival (of 20)	Bud growth
	Temp. (°C)	Time (hrs)		
Preconditioned[a]	Control		20	17/20
	48	2	20	18/20
	48	3	18	15/18
	50	2	20	15/20
	50	3	16	6/16
Nonpreconditioned[b]	Control		19	15/19
	48	2	15	7/15
	48	3	9	7/9
	50	2	2	1/2
	50	3	0	

a. Budwood was preconditioned for 28 weeks at temperatures of 30–31° minimum and 38–42° C maximum.
b. Normal temperatures were 20–22° minimum and 27–33° C maximum.

for 3, 3½, and 4 hours. Four buds from treated shoots and untreated controls were promptly grafted into each of 3 indicator seedlings of Mexican lime for detection of vein-enation and tristeza viruses, sweet orange for concave-gum virus, and Etrog citron for exocortis virus. For detection of stubborn virus, 2 side grafts plus 2 buds were grafted into each of 3 Sexton tangelo seedlings. Indicator plants were observed for 15 months, and the results of tissue survival and virus transmission for stubborn and vein-enation viruses are given in Table 2.

TABLE 2. GRAFT SURVIVAL AND VIRUS TRANSMISSION FROM PRECONDITIONED BUDWOOD TREATED IN HOT WATER AT 50° C

Virus and host	Hrs. at 50° C	Graft survival (of 12)	Diseased plants (of 3)
Vein enation	0	12	3
Mexican lime	3	4	0
	3 1/2	0	0
	4	0	0
Stubborn (A)	0	12	3
Sweet orange	3	12	0
	3 1/2	11	1
	4	12	0
Stubborn (B)	0	12	3
Sweet orange	3	12	0
	3 1/2	12	0
	4	11	0
Stubborn (C)	0	12	3
Sweet orange	3	4	0
	3 1/2	4	0
	4	1	0

Exocortis, concave-gum, and tristeza viruses survived 4, 4, and 3½ hour treatments, respectively, at 50°C. Exocortis and tristeza viruses were transmitted even though the inoculum buds of mandarin and lime were killed, respectively, by 4- and 3½-hour treatments. Nonpreconditioned stubborn-infected sweet orange did not survive heat treatment. Vein-enation virus was inactivated in budwood treated 3 hours at 50°C, and serial inoculations by bud grafts from 2 symptomless Mexican lime plants caused no reaction.

Stubborn virus in sweet orange budwood from all 3 donor plants appeared to be inactivated in 3–4 hours at 50°C except in one instance (Table 2). Graft survival from treated shoots of preconditioned stubborn plants was 78, 75, and 67 per cent, respectively, for treatments of 3, 3½, and 4 hours at 50°C; all shoots from the nonpreconditioned stubborn plants were killed by hot water treatments. All grafts from the unheated stubborn control shoots survived and all indicator plants inoculated by them developed symptoms.

EXPERIMENT 3: DRY AND MOIST HOT AIR TREATMENTS OF STUBBORN-INFECTED BUDWOOD.—Greenhouse-grown, preconditioned shoots were compared with field-grown shoots for tolerance to hot air and to hot moist air. Five sources of stubborn-infected budwood were used: A, B, and C were greenhouse-grown budlings of navel and 2 Madam Vinous sweet orange preconditioned 24 weeks at temperatures similar to those in experiment 1; D and E were stubborn-infected field trees, a Valencia and a navel orange. In May 1967, budwood from each donor was cut into 10-cm lengths and treated in dry and in moist air for 3, 4, and 5 hours at 50°C. For treatment in moist air, bundles of bud-

wood were held upright in a sealed plastic container with 4 mm of water on the bottom; the container was placed in a dry hot air chamber. Temperatures inside the hot air chamber and sealed plastic container behaved approximately the same with the exception of a 20-minute delay in bringing the temperature from 43° to 50°C inside the plastic container after insertion of the budwood.

Treated and untreated budwood was kept at room temperature for a few hours, refrigerated at 7°C overnight in polyethylene bags, and graft-inoculated into Madam Vinous sweet orange indicator seedlings, 2 side grafts and 4 buds per seedling being used. Graft survival was recorded at 4 weeks, and stubborn evaluations were made over a 7-month period.

Graft survival of preconditioned shoots treated for 0, 3, 4, and 5 hours at 50°C was 95, 72, 61, and 56 per cent, respectively, in dry air and 95, 84, 72, and 56 per cent, respectively, in moist air, indicating that moisture had little effect on survival of preconditioned budwood. However, stubborn virus appeared to be inactivated by the 4- or 5-hour treatments in moist air, but survived 5 hours in dry air. The results indicate that treatment periods in excess of 5 hours at 50°C in moist air should be tried.

Budwood from the 2 field trees did very poorly after treatment in dry or moist air at 50°C. Field budwood treated in dry air for 0, 3, 4, and 5 hours at 50°C showed 94, 17, 8, and 17 per cent survival, respectively. There was no survival of field budwood treated in moist air at 50°C.

EXPERIMENT 4: PROLONGED MOIST HOT AIR TREATMENT OF MEYER LEMON FOR ELIMINATION OF TATTERLEAF-CITRANGE STUNT VIRUS.—This experiment was performed to determine the effect of prolonged treatments at 50°C on Meyer lemon and to explore the possibility of eliminating the tatterleaf–citrange stunt virus complex from a tristeza-virus-free Meyer lemon. The source plant was a Meyer lemon indexed and found to be free of tristeza, seedling-yellows, vein-enation, psorosis, and exocortis viruses, but to contain the tatterleaf–citrange stunt virus complex. The plant was preconditioned for 10 weeks from May through July 1967 in a warm room where maximum temperatures reached 42–44°C. Newly hardened shoots from the upper portion of the plant were treated in moist air as described in experiment 3. Two bud sticks 10 cm long were randomly selected for each treatment except that 4 sticks were reserved for untreated controls. Treatment times at 50°C were 0, 3, 4, 5, 6, 7, 11, and 22 hours. Soon after treatment, 2 buds from each treatment were grafted to each of 4 Troyer citrange seedlings. After 4 weeks, the Troyer seedling was cut back to force new shoots, and observations for citrange-stunt symptoms were made for 2 years. Results are shown in Table 3.

All buds of Meyer lemon survived all treatments, even that of 22 hours

at 50°C. Meyer lemon buds were forced on their Troyer stocks and the budlings were serially indexed twice over a 2-year period to Troyer citrange and *Citrus excelsa* West. The results showed that 10 Meyer lemon propagations were free of tatterleaf–citrange stunt virus. One of the 8 untreated controls was apparently free of virus, indicating possible inactivation of virus during preconditioning.

TABLE 3. BUD SURVIVAL AND INACTIVATION OF TATTERLEAF-CITRANGE STUNT VIRUS IN PRECONDITIONED MEYER LEMON BUDWOOD TREATED IN MOIST AIR AT 50° C

Hrs. at 50°C	Buds surviving (of 8)	Symptomless citrange indicators (of 4)
0	8	0
0	8	1
3	8	0
4	8	1
5	8	1
6	8	1
7	8	3
11	8	0
22	8	3

Similarly treated preconditioned sweet orange budwood infected with stubborn virus survived moist air treatments of 3–11 hours with resultant inactivation of stubborn virus in 20 of 24 plants, whereas nontreated budwood transmitted stubborn in all 8 control plants. The 22-hour treatment was not used on sweet orange budwood.

Discussion and Conclusion

Preconditioning citrus plants to warm temperatures prior to heat treatment enabled their excised shoots to tolerate heat treatments in excess of those previously reported. For example, the maximum tolerance of sweet orange budwood to agitated hot water at 50°C was reported to be about 15 minutes by Grant et al. (6) and about 60 minutes by Fawcett and Cochran (3) and Price and Knorr (9). Rossetti et al. (11) noted that sweet orange or Rangpur lime buds did not survive 1 hour at 50°C, and Schwarz and Green (12) found the maximum tolerance of sweet orange budwood to be between 1 and 2 hours at 50°C. With preconditioning we have extended survival time at 50°C to 4 hours in hot water and 11 hours in moist air. In contrast, budwood from our nonpreconditioned sweet orange plants rarely survived 2 hours in water at 50°C. There is some evidence in experiment 4 that preconditioning alone for a period of 10 weeks at warm temperatures may have inactivated the tatterleaf–citrange stunt complex in some shoots of Meyer lemon.

Preconditioning at high temperatures is a valuable preliminary procedure for thermotherapy of citrus. Our results indicate that stubborn virus in citrus budwood is usually inactivated in 3–11 hours at 50°C, either in water or moist air. However, on occasion, stubborn virus did survive heat treatment (experiments 2 and 3). Vein-enation virus in budwood was inactivated in 3 hours by water at 50°C, and the tatterleaf–citrange stunt virus complex was inactivated in some buds of Meyer lemon in 4–22 hours by moist air at

50°C. Tristeza, concave-gum, and exocortis viruses in budwood were not inactivated in 4 hours by water at 50°C.

The survival of all Meyer lemon buds treated 22 hours at 50°C in moist air suggests that preconditioned budwood of some citrus species may tolerate even longer exposure periods at 50°C or higher treatment temperatures.

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