HEAT THERAPY OF VIRUS DISEASES OF PERENNIAL PLANTS

By George Nyland, Department of Plant Pathology
and
A. C. Goheen, Agricultural Research Service,
U. S. Department of Agriculture
University of California, Davis, California

Introduction

Clean stocks of perennial crop plants are important for optimum production, and are even more essential for physiological studies since diseased plants differ greatly from healthy plants in their physiology. For example, infection with the grapevine leafroll virus causes a deficiency of cation uptake in leaves. As a result, early investigators of leafroll, considering it a disorder in mineral nutrition, were baffled by the lack of response to fertilizers. Cook & Goheen (38) showed that the mineral-deficiency symptoms were a response to a virus disease. Investigators need no longer work with perennial plants of unknown virus content since clean stocks can be obtained by heat therapy, alone or combined with meristem-tip culture. foliage

Heat is an important therapeutic agent for treating diseases in plants. Over 100 years ago, Scots gardeners immersed bulbs in hot water before planting, thus being the first known to use heat for therapy of plants (209). The therapeutic benefits of heat used against a specific disease became widely known with work of Jensen (110). Wilbrink (204) suggested that Jensen's work may have prompted Sayer to try hot-water treatment of sugarcane infected with sereh disease. Although that treatment was not used extensively in treating sereh-diseased cane setts, it came into widespread use when sugarcane chlorotic streak (127) and, later, ratoon stunt (181) appeared.

Kunkel found that peach yellows was cured by either dry heat or hot-water treatment (112), and used both methods in curing 11 diseases of the yellows group, including aster yellows (115–117). No virus diseases other than those of the yellows group were successfully cured by heat until Kassanis (100) reported curing potato leafroll. If Blodgett (14) had chosen potato leafroll instead of potato mosaic for heat treatment, he would have antedated Kunkel by 13 years, and Kassanis by 26. His maximum treatment of 35° C for 4 months would most certainly have cured potato tubers of leafroll although it did not inactivate potato mosaic virus, probably potato virus X (PVX). Recent reports (42) of the association of mycoplasma organisms with diseases of the yellows group could mean that diseases spread by leafhoppers, and perhaps some others that also are easily hot-water-la-

bile *in vivo*, have similar etiologic agents. This would mean that no viruses *sensu* Bawden (7) are hot-water-labile *in vivo* at 50 and 52° C for 20 to 30 min. We are not aware of any unequivocal evidence of the virus nature of any disease that is easily cured by hot-water treatments.

Several earlier reviews treat the subject of heat therapy of virus diseases or closely related subjects. Some cover heat therapy of plant virus diseases directly (13, 90, 102–105, 110, 125, 126, 139); others cover heat therapy of the virus diseases of a specific group of crop plants (79, 145). Still others are concerned with virus-disease control, and consider heat therapy an important tool for curing plants (72, 82, 91, 92, 170, 187). Finally, some reviews cover the general therapy of plant diseases, providing sections on heat therapy of plant viruses (57, 182). Related reviews treat the subject of the effect of temperature on plant disease and disease control (3, 4, 6, 206, 207), and the physiological responses of biological systems to temperature (59, 61, 96). Heat therapy is used frequently along with meristem culture (135, 159, 160). Heat therapy has also become a subject for sections in books on plant viruses (7, 24).

Heat therapy is in progress in several locations, and new issues of many journals contain references to successful treatment of additional viruses. According to Kristensen (110), approximately 1000 papers on heat therapy or related subjects appeared before 1966. The number of viruses successfully inactivated *in vivo* has steadily increased. Fifteen were reported by 1950, 75 by 1960, and 100 by 1966 (90). We report about 120 to the present. The space available does not permit listing all of the papers that have appeared on this subject or a review of heat treatment of viruses in seed.

We review the methods and results of heat treatment and factors affecting them and point out the important uses of heat therapy. We attempt to group viruses with similar heat reactions on the basis of the incomplete data available in the literature. We discuss thermal constants and the mechanism of inactivation, and suggest that the heat stability of plant viruses is indicated more meaningfully by half-life *in vivo* at 38° C (when it can be obtained) than by the thermal inactivation point. Such precise data might reveal more clearly the relationships and mechanisms involved in inactivation.

Methods Used in Heat Treatment

For asexually propagated plants that are completely infected with virus there are five methods reported to obtain virus-free explants: (*a*) nucellar embryony, which is useful in citrus (201); (*b*) chemotherapy (72); (*c*) meristem (135, 160) or tip culture (88); (*d*) leaf removal, as can be practiced only in the case of Abutilon mosaic virus, so far as is known (90, 109); and (*e*) heat therapy. The most important of the five methods listed is heat therapy. Heat is applied either by hot water in controlled-temperature tanks or by hot air in controlled-temperature chambers. To obtain virus-free explants from plants infected with viruses not completely labile with heat

treatments alone, heat therapy is frequently combined with meristem and tip culture.

Hot water and moist air.—Hot water was used earlier than hot air in treatments, and is used extensively with sugarcane diseases. Early workers established the desirability of rapid circulation of water around the individual pieces of plant tissue (68). They also revealed the greater tolerance of host tissue to hot moist air treatments, in which heat is applied in water-saturated warm air (119, 181). Baker (3) suggests that some of the causes of damage to plants or plant parts treated in water at temperatures necessary for therapy are leaching, water-soaking, and asphyxiation of host tissues.

Hot-water baths and chambers for applying moist heat vary in size from laboratory models to equipment large enough to treat a ton or more of sugarcane at one time. Control of temperature and time is very important because of the small spread between the heat inactivation point of the virus and the maximum tolerance of the host.

Hot-air treatments.—Chambers used to treat infected plants in containers for days or weeks at 35 to 40° C have varied from boxes slightly larger than a dog kennel (79) to walk-in rooms capable of treating large numbers of plants (143). Chambers described in earlier reports (17, 51, 102, 112, 152) used natural light. Natural light is ideal if supplemented by artificial light during short days. Artificial light alone is also satisfactory if of sufficient intensity. Either glass or plastic is used for the tops and sides of heat chambers exposed to natural or artificial light. A sophisticated chamber permits indefinite treatment of some plant species at 38° C (65, 178, 186). The improvement in both equipment and techniques has permitted treatment of strawberry plants and chrysanthemums for up to 8 months (21, 132), and citrus for 3 years (188). Some plants grow even better in the heat chamber than in the greenhouse (76). Satisfactory heat-treatment chambers can be constructed at a modest cost (49).

Preconditioning increased the survival time of plants in the heat room and of plants or plant parts in hot water. Thus, exposing plants to 27 to 35° C for a week or two prior to exposure at 38° C increased survival time in the heat room (51, 145, 203). Also beneficial is a gradual increase in the temperature (16, 79). Factors favoring survival of plants or plant parts under heat treatment are high carbohydrate reserve, maturity of tissues, partial dehydration that accompanies storage, previous growth at high temperature, and low humidity (3, 65).

The temperature in the root zone and in the plant during treatment is usually lower than the air temperature. Lower root temperature favors survival of the plants and does not seem to interfere with therapy (47, 107, 131, 144, 152). For this reason, some prefer clay pots to metal or plastic pots (131, 153). The temperature may be 5.5° C lower in the root zone of strawberry plants in clay pots than in the air (131, 132) but in plastic pots there is less difference. The temperature in the tissue of the tops of the plants may average 6° C lower than the air temperature; the difference in

temperature among individual plants may be 5° C (49). In hot-air treatments for short periods, the temperature at the center of scions placed in an oven at 75° C reached 50° C in 10 min and did not reach 55° C after 20 min (106).

Plant survival is increased by intermittent application of heat. Heating plants on alternate days did not inactivate peach yellows (112). Hamid & Locke (71), however, successfully treated eye-pieces of potato tubers infected with leafroll virus at 40 or 45° C for 2 hr alternating with 22 hr at room temperature for 8 weeks, or 2 weeks, respectively. The thermal-death-time of potato tuber eye-pieces at a constant temperature of 34, 37, or 40° C was less than that of the virus. Larson (118) found that chrysanthemum plants infected with virus B survived 4 weeks at a constant temperature of 38° C, but survived for 12 weeks at a daily temperature cycle of 38 or 40° C for up to 16 hr, and 20° C for 8 hr. He could not propagate tips from plants held constantly for 4 weeks at 38° C. The percentage of tips free of virus was high only when the high temperature was held for at least 16 hr daily. Citrus stubborn virus was not inactivated when exposed *in vivo* for 37 days at alternating temperatures of 44.5° C for 9 hr and 30° C for 15 hr (148).

Temperatures fluctuating between 35 and 43° C have been reported as more favorable for plant survival than constant temperature at 38° C (131, 132), but there is little information on the effect of intermittent treatments or fluctuating temperature on the efficiency of virus inactivation. Weil (202) found that the total time required to inactivate TMV *in vitro* was less with separate intermittent exposures to 90° C than with continuous exposure. This may also be true with *in vivo* treatments of some viruses. Hamid & Locke (71) with intermittent exposure inactivated potato leafroll in 4 days at 40° C, whereas with continuous exposure Rozendaal et al. (167) needed 12 days at the same temperature. Comparison of continuous and intermittent treatment of chrysanthemums may show a similar difference if this is expressed as the size of tips that can be taken (70, 118).

The survival of infected plants in the heat chamber is also affected by plant age, length of time since transplanting into containers, and seasonal effects. A few specific examples can be mentioned. Kunkel (112) noted that older trees survived better than younger trees. Plants well established in the containers survived better than those recently transplanted (16, 102, 144, 152, 198). Late summer or autumn treatment is preferred for grapes (64), potato tubers (175), and strawberries (17). Grapes treated for 60 days in autumn produced 3 to 5 times as many tips as grapes treated in spring. Fruit-tree species are best treated in late summer (145) if lateral buds are removed during or after treatment (144), or if dormant buds are placed in seedlings prior to treatment (93). If shoot tips produced during treatment are to be removed, however, Campbell (27) preferred to treat in the spring. April or May is the time preferred by some to treat chrysanthemums (111), but summer is also recommended (83).

Plants in the heat chamber usually show temporary damage or abnor-

malities. Some plants develop abnormal color and shape of leaves (186, 187) or fail to develop normal storage roots (74). Other temporary changes were devernalization and fasciation in chrysanthemums (21).

Special techniques in heat therapy.—Special techniques for therapy have been developed for some species, to be used with or without heat treatment (87, 93). Galzy (52, 53) heat-treated explants of grape growing on agar at 35° C, studied the effect of virus on rooting and established plants free of fanleaf by removing tip cuttings from the treated explants. The method should be applicable to other species and lend itself to various physiological studies. Rich (164) removed eyes, both before and after heat treatment, from potato tubers infected with PVX, and implanted them in tubers of a variety immune to PVX. In both cases he obtained plants free of virus. Mellor & Fitzpatrick (131) excised and rooted axillary buds that developed on the older parts of the crowns of strawberry plants after or during heat treatment.

Virus therapy is assisted by combining chemical treatments with heat. One treatment aimed at reducing the respiration rate with KCN (39), and another aimed at inhibiting the virus with malachite green (89) in the water in which plant parts were treated. Thiouracil, 2,4-D, and indoleacetic acid applied to carnation plants or to the medium used for meristem culture, gave beneficial results (159). Bolton (16) applied 150 ml of one per cent potassium permanganate solution each week to strawberry plants during treatment at 39° C or above to control root-disease organisms.

The success of heat therapy in air depends in most cases upon removal of a small to large portion of the treated plant after the prescribed exposure to high temperature. Combining heat treatment with meristem culture or tip removal increased the efficiency of therapy (10, 27, 45, 70, 149, 159). Among the easiest methods to apply, where they will work, are removing lateral buds after treatment and placing them in clean seedlings in a nursery, or simply dividing the stems of treated plants into cuttings and rooting them. Explants may or may not be free of virus, so they require indexing.

Heat Stability and Virus Grouping

Kassanis suggested (101) that heat treatment might be useful in grouping viruses when more is known about the mechanism of action. Our attempt to do so might be considered a beginning, though still premature. We have tabulated (Tables I, II) the known facts published on heat therapy and listed the last two of the four groups of Gibbs' (58) cryptograms to relate these facts to other properties of the viruses. We used the Kew list (128) for virus names. For each virus we list only a single reference to the minimum temperature-time treatment that was effective.

Viruses of the yellows group are placed together in the first section in Table I. A few viruses are included that resemble yellows but their vectors are not known to be leafhoppers. Most within this group are inactivated at 10 to 20 min at about 50° C. Some require longer times. Times reported are

TABLE I

PLANT VIRUSES INACTIVATED *in vivo* BY HEAT

Group and virus name	Cryptogram[a]	Host treated	Treatment			Authority
			Temp. °C	Time	Type	
YELLOWS TYPE						
Aster yellows	*/*:S, I/Au	Vinca	45	2.5 hr	W[b]	114
			42	2 wk	A	114
Bayberry yellows	*/*:S/*	Vinca	42	6 da	A	162
Cherry little cherry	*/*:S/Au	Prunus	37.5	3 wk	A	147
Chrysanthemum flower distortion	*/*:S/Au	Chrysanthemum	35	2 mo	A	22
Citrus yellow shoot		Citrus	50	40 min	A	119
Clover dwarf	*/*:S/Au	Vinca	40	10 da	A	199
Clover phyllody	*/*:S/Au	strawberry	41	14 da	A	155
		Vinca	40	10 da	A	199
Clover wound tumor	S/S:S, I/Au	sweet clover	40	12 da	A	171
			14	8 wk	A	171
Crimean yellows	*/*:S/Au	Vinca	40	10 da	A	199
Delphinium yellows	*/*:S/Au	Vinca	41	3 wk	A	155
Grapevine flavescence doree	*/*:S/Au	grape	30	3 da	W	32
Guatemala grass spikiness	*/*:S/*	Guatemala grass	52	20 min	W	137
Lucerene witches' broom		Vinca	40	7 da	A	117
Mulberry dwarf	*/*:S/Au	mulberry	55	40 min	W	189
			50	1 da	A	189
Opuntia witches' broom		Opuntia	45	5 hr	W	130
			45	15 da	A	142
Parastolbur	*/*:S/Au	Vinca	40	10 da	A	199
Peach phony	*/*:S/Au	peach	48	40 min	W	94
Peach rosette	*/*:S/Au	peach	50	10 min	W	112
			35	2 wk	A	112
Peach x-disease	*/*:S/Au	peach	50	6 min	W	73
Peach yellow leafroll	*/*:S/Au	peach	50	10 min	W	141
Peach yellows	*/*:S/Au	peach	50	10 min	W	112
			35	2 wk	A	112
Pennisetum streak	*/*:S/*	Pennisetum	52	20 min	W	26
Potato witches' broom	*/*:S/Au	potato tubers	36	6 da	A	115
		Vinca	42	13 da	A	115
Rubus stunt	*/*:S/Au	Rubus	45	1 hr	W	196
			37	21 da	A	95
Stolbur	*/*:S/Au	Vinca	40	10 da	A	199
Sugarcane chlorotic streak	*/*:S/Au	sugarcane	52	20 min	W	127
			54	8 hr	A	1
Sugarcane grassy shoot	*/*:S/(Ap)	sugarcane	50	30 min	W	174
			54	8 hr	A	174
Sugarcane sereh	*/*:S/*	sugarcane	45	30 min		
			+52	30 min	W	204
Sugarcane whiteleaf	*/*:S/Au	sugarcane	54	50 min	W	120
			54	8 hr	A	120
Vaccinium (cranberry) false-blossom	*/*:S/Au	cranberry	42	8 da	A	116
Vaccinium stunt	*/*:S/Au	blueberry	52	2 hr	A	185

[a] Third and fourth pairs of Gibbs (58), third pair = outline of particle/outline of "nucleocapsid"; S = spherical, E = elongated with parallel sides, ends not rounded, U = elongated with parallel sides, ends rounded. Fourth pair = kinds of hosts infected/kinds of vector; F = fungus, I = invertebrate, S = seed plant/Ac = mite, Al = white fly, Ap = aphid, Au = leaf plant, or tree hopper, Cc = mealy bug, Fu = fungus; * = unknown and ( ) = doubtful.

[b] W = water; A = air.

TABLE I—(*Continued*)

Group and virus name	Cryptogram[a]	Host treated	Treatment			Authority
			Temp. °C	Time	Type	
HOT WATER LABILE—*Not sap transmissible*						
Cherry necrotic rusty mottle	*/*:S/*	cherry	50	10 min	W	143
Hibiscus leafcurl	*/*:S/AL	Hibiscus	40	25 min	W	136
			35	25 da	A	136
Potato leafroll	S/S:S, I/Ap	potato tubers	50	17 min	W	140
			36	20 da	A	100
Strawberry complex		strawberry	43	30 min	W	134
HOT WATER LABILE—*Sap transmissible*						
Hop nettlehead complex		hop	45	5 min	W	62
Prune dwarf	S/S:S/*	plum	35	30 hr	W	36
		peach	38	17 da	A	144
Prunus necrotic ringspot	S/S:S/*	cherry	35	36 hr	W	44
			38	17 da	A	144
Cherry line pattern complex		cherry	45.5	3 hr	W	69
Sugarcane ratoon stunting		sugarcane	50	2 hr	W	181
			54	8 hr	A	180
ONLY HOT AIR LABILE—*Easily inactivated*						
Abutilon mosaic	(S)/*:S/Al	Abutilon	37	21 da		102
Alfalfa mosaic	U/U:S/Ap	alfalfa	36	8 da		50
Apple chlorotic leafspot	E/E:S/*	apple	38	7 da		203
Apple leaf pucker	*/*:S/*	apple	38	7 da		203
Apple (Malus) platycarpa dwarf	*/*:S/*	Malus	37	20 da		27
Apple (Malus) platycarpa scaly bark	*/*:S/*	Malus	37	20 da		27
Apple rubbery wood	*/*:S/*	apple	38	7 da		203
Apple Spy 227 epinasty and decline	*/*:S/*	apple	38	7 da		203
Apple stem pitting	E/E:S/*	apple	38	7 da		203
Arabis mosaic	S/S:S/Ne	*Nicotiana clevelandii*	38	21 da		81
Bean yellow mosaic	E/E:S/Ap	sweet clover	10	6 wk		9
Black currant reversion	*/*:S/Ac	Ribes	34	20 da		28
Carnation ringspot	S/S:S/*	carnation	37	21 da		149
Carnation streak	*/*:S/*	carnation	37	28 da		192
Cassava mosaic	*/*:S/Al	carnation	39	28 da		35
Chrysanthemum green flower	*/*:S/*	Chrysanthemum	38	28 da		183
Chrysanthemum ringspot	*/*:S/*	Chrysanthemum	36	21 da		83
Chrysanthemum stunt (English strain)	*/*:S/*	Chrysanthemum	36	21 da		83
Citrus greening	*/*:S/Ps	citrus	40	28 da		121
Citrus infectious variegation	S/S:S/Ap	citrus	38	30 da		123
Citrus stubborn	*/*:S/*	citrus	51	1.5 hr		148
Citrus tristeza	E/E:S/Ap	citrus	39	28 da		41
Clover (white) new		*N. clevelandii*	38	28 da		86
Clover yellow vein	E/E:S/Ap	*N. clevelandii*	38	4 wk		84
Cucumber mosaic	S/S:S/Ap	Passiflora	37	14 da		190
Fig mosaic	*/*:S/Ac	fig	37	24 da		31
Gooseberry veinbanding	*/*:S/Ap	Ribes	35	14 da		98
Grapevine fanleaf (strain of Arabis mosaic)	S/S:S/Ne	grape	35	21 da		53
Mushroom, 1 & 2	S/S:Fu/*	mushroom	33	?		54
Mushroom, 3	U/U:Fu/*	mushroom	33	14 da		55
Peach (Muir) dwarf	*/*:S/*	peach	38	17 da		144
Pear bark necrosis	*/*:S/*	pear	37	28 da		154
Pear stony pit	*/*:S/*	pear	70	10 min		36

TABLE I—(*Continued*)

Group and virus name	Cryptogram[a]	Host treated	Treatment			Authority
			Temp. °C	Time	Type	
Pear vein yellows	*/*:S/*	pear	37	28 da		154
Potato A	E/E:S/Ap	potato	35	14 da		195
Potato Y	E/E:S/Ap	potato	38	7 da		194
Prunus necrotic ringspot	S/S:S/*	Prunus	38	17 da		144
Prunus necrotic ringspot (apple mosaic strain)	S/S:S/*	Malus	37	20 da		193
Raspberry mosaic heat-labile components	*/*:S/Ap	Rubus	38	4 da		37
Rose mosaic	*/*:S/*	rose	35	28 da		88
Rose yellow mosaic	*/*:S/*	rose	38	14 da		198
Strawberry crinkle	*/*:S/Ap	strawberry	38	3 da		10
Strawberry latent C	*/*:S/Ap	strawberry	35	22 da		122
Strawberry mild yellow edge	*/*:S/Ap	strawberry	37	26 da		153
Strawberry mottle	*/*:S/Ap	strawberry	37	7 da		17
Strawberry veinbanding	*/*:S/Ap	strawberry	42	10 da		15
Strawberry, unspecified		strawberry	37.5	10 da		18
Tobacco ringspot	S/S:S/Ne	cowpea, tobacco	36	28 da		77
Tomato aspermy	S/S:S/Ap	Chrysanthemum	38	14 da		111
Tomato ringspot (Peach yellow bud mosaic strain)	S/S:S/Ne	peach	38	21 da		146
Tomato spotted wilt	S/S:S/Th	tomato	38	28 da		79
Turnip mosaic	E/E:S/Ap	horseradish	37	21 da		89
ONLY HOT AIR LABILE—*Difficult to inactivate*						
Broad bean mottle	S/S:S/*	*N. clevelandii*	38	6 wk		86
Carnation etched ring	S/S:S/*	carnation	38	6 wk		20
Carnation I.R.	S/S:S/*	*N. clevelandii*	38	6 wk		86
Carnation latent	E/E:S/Ap	carnation	38	8 wk		20
Carnation mottle	S/S:S/I	carnation	40	6 wk		159
Carnation vein mottle	E/E:S/Ap	carnation	40	8 wk		159
Cherry (sour) green ring mottle	*/*:S/*	sour cherry	38	6 wk		146
Chrysanthemum B	E/E:S/Ap	Chrysanthemum	37	2 mo		70
Chrysanthemum rosette	*/*:S/*	Chrysanthemum	35	2 mo		23
Citrus exocortis	*/*:S/*	lemon	38	33 wk		188
Citrus psorosis	*/*:S/I	citrus	35–43	12 wk		66
Citrus yellowing		lemon	38	22 wk		188
Grapevine asteroid mosaic	*/*:S/*	grape	38	6 wk		64
Grapevine corky bark	*/*:S/*	grape	38	14 wk		64
Grapevine leafroll	*/*:S/*	grape	38	8 wk		64
Grapevine yellow vein (strain of ringspot)		grape	38	6 wk		63
Hydrangea ringspot	E/E:S/*	hydrangea	35	12 wk		19
Peach stubby twig	*/*:S/*	peach	38	5 wk		146
Pelargonium leafcurl	S/S:S/*	*N. clevelandii*	37	30 da		80
Plum pox	E/E:S/Ap	Prunus	38	39 da		107
Poplar mosaic	E/E:S/*	poplar	38	6 wk		11
Potato S	E/E:S/Ap	potato plants	35	6 wk		178
Potato X	E/E:S/(Fu)	potato plants	35	15 wk		178
Strawberry witches' broom	*/*:S/*	strawberry	35–46	8 wk		131
Sweet potato internal cork	E/E:S/Ap	sweet potato plants	38	3 mo		74
Sweet potato leafspot	*/*:S/Ap	sweet potato plants	38	1 mo		76
Sweet potato yellow dwarf	*/*:S/Al	sweet potato plants	38	1 mo		76
Turnip crinkle	S/S:S/Cl	Brassica	38	6 wk		86

TABLE II

VIRUSES NOT INACTIVATED BY HEAT TREATMENT *in vivo*

Virus name	Cryptogram	Host treated	Treatment			Authority
			Temp. °C	Time	Type	
Apple (Virginia crab) decline		apple	38	4.5 wk	A[a]	203
Cacao swollen shoot	U/*:S/Cc	Cacao budwood	50	25 min	W	138
Cherry mottle leaf	*/*:S/Ac[b]	cherry	38	5 wk	A	146
Chrysanthemum D	*/*:S/*	Chrysanthemum	37	4 wk	A	83
Chrysanthemum E	*/*:S/*	Chrysanthemum	36	4 wk	A	83
Chrysanthemum stunt (American strain)	*/*:S/*	Chrysanthemum	38	12 wk	A	118
Chrysanthemum vein mottle	E/E:S/Ap	Chrysanthemum	36	4 wk	A	83
Citrus xyloporosis	*/*:S/*	Citrus	35	75 da	A	67
Dioscorea green-banding	*/*:S/Ap	Dioscorea tubers	50	1 hr	W	168
			37	2 wk	A	168
Mulberry mosaic	*/*:S/Ap	mulberry	40	21 da	A	163
Narcissus yellow stripe	E/E:S/Ap	Narcissus	37	21 da	A	25
Peach calico	*/*:S/*	peach	37	40 da	A	33
Peach mosaic	*/*:S/Ac	peach	50	32 min	W	113
			35	30 da	A	113
Plum bark split	*/*:S/*	plum	37	39 da	A	45
Potato aucuba mosaic	E/E:S/Ap	potato tubers	38	5 wk	A	166
Potato mop top virus	E/E:S/Fu	potato	37	7 wk	A	99
Potato spindle tuber	*/*:S/*	potato tubers	35	39 da	A	46
Quince sooty ringspot	*/*:S/*	quince	50	8 min	W	154
			37	28 da	A	154
Raspberry leaf curl	*/*:S/Ap	raspberry	37	28 da	A	177
Raspberry mosaic heat stable components	*/*:S/Ap	raspberry	37	90 da	A	176
Raspberry vein chlorosis	*/*:S/Ap	raspberry	50	?	W	34
Raspberry yellows	*/*:S/*	raspberry	50	?	W	34
Strawberry necrotic shock	*/*:S/*	strawberry	20–44	6 mo[c]	A	48
Sweet potato russet crack	*/*:S/*	sweet potato	38	4 mo	A	75
Tobacco rattle virus	E/E:S/Ne	potato tubers	38	5 wk	A	166
Vaccinium (blueberry) ringspot	*/*:S/*	Vaccinium	60	1 hr	W	184
(=tobacco ringspot?)			38	?	A	184

[a] W = water; A = air.
[b] Personal communication, L. S. Jones, USDA Entomology, Riverside, California.
[c] Treated in the field at Meloland, California.

often minimum times tested and not necessarily the thermal death-point. This is true for cherry little cherry, chrysanthemum flower distortion, Crimean yellows, delphinium yellows, parastolbur, and stolbur. In some cases water treatments were not tried, only air. Mycoplasma organisms have been associated with several (42, 120) of the diseases listed in this part of Table I. Mycoplasmas associated with animal diseases are also easily heat-inactivated (169).

The second section of Table I includes four diseases easily inactivated that are not sap- or leafhopper-transmitted, and have not yet been proved to

be viruses. On the basis of heat sensitivity the etiologic agent of these diseases could be similar to that of yellows.

The third section of Table I includes five diseases also easily inactivated for which definite virus particles have been described, although some question still remains for ratoon stunting. All are sap-transmissible.

The fourth section of Table I includes viruses designated as easily inactivated on the arbitrary basis that inactivation in less than 29 days was sufficient to permit propagation of explants free of infection (half-life less than 29 days at *ca* 38° C). Insect vectors and virus particles have been described for some in this group but not for others.

The last section of Table I lists viruses that we classify as difficult to inactivate (half-life greater than 28 days at *ca* 38° C). Treatment periods shown were the minimum required to obtain at least some explants free of infection. Almost all treatments in this group were combined with either meristem culture or removal of tips or axillary buds.

The basis for successful heat inactivation *in vivo* is the difference between the host and virus in tolerance to high temperature. There might be an equal difference in tolerance to low temperature between the host and virus that could be utilized in therapy. Two quite different viruses—clover wound tumor and bean yellow mosaic in clover—were inactivated when infected plants were grown at temperatures near minimum for plant growth. Plants infected with potato virus Y were also reported to be cured at low temperature (90). Additional diseases, both yellows and nonyellows, might respond to low-temperature therapy, but we are not aware of any others that were tested.

Table II includes some viruses that have been tested many times but have resisted all attempts to inactivate them, as well as others that have not been adequately tested. The exceptional tolerance to heat of some suspected viruses such as chrysanthemum stunt, sweet potato russet crack, strawberry necrotic shock, and the heat-stable components of raspberry mosaic suggests that these viruses may exist in the host as nucleic acid, as has been shown for citrus exocortis (172). This virus is extremely heat stable but was inactivated after treatment at 38° C for 33 weeks (188).

Virus Shape and Stability

As apparent in the tables, the morphology of virus particles is not associated consistently with ease or difficulty of inactivation by heat *in vivo*. Up to 1964, heat therapy was successful only with spherical viruses or with viruses of unknown particle morphology (102, 104). Among the viruses grouped under "easy to inactivate" for which particle morphology is known, 10 are rod-shaped and 13 are spherical. In the group designated "difficult to inactivate," 9 are rod-shaped and 6 are spherical. Of the viruses not yet inactivated (Table II), 6 are rod-shaped and 19 are of unknown morphology.

Probably all viruses can be inactivated *in vivo* with the right combina-

tion of temperature and time and attention to all factors favoring plant survival (8).

Indexing for Proof of Virus Inactivation

Virus inactivation is assessed either by observation of treated plants or explants, or by indexing procedures. Indexing by graft inoculation is more reliable than assay by sap transmission, insects, electron microscopy, or serology (37, 152, 153, 178). Heat-tolerant strains or the original virus may remain undetected during one or more index tests after treatment (34, 79, 153, 156). Inactivation of a virus in different hosts may vary within an experiment and between experiments (152, 153, 203). In most cases, observation alone cannot be considered proof of inactivation (40, 79, 150, 159, 178, 179).

Viruses like PVS in potatoes are particularly difficult to detect since they can be demonstrated only by serology or electron microscopy (178). In indexing for PVX and PVS (178), the virus was assumed to be PVS when flexuous rods were present and the PVX test on *Gomphrena* was negative. Half of the plants that were negative for PVS in electron microscopy still indexed positive for PVX serologically.

Cross-protection tests and reinoculation of treated plants are also used for proof of virus inactivation (114, 166).

Thermal Constants and the Mechanism of Heat Inactivation

The usual thermal constant given for plant viruses is the thermal inactivation point [the temperature required to inactivate virus in an aliquot of infected pressed sap in 10 min *in vitro* (97)]. With proper care, this point can be determined for viruses that are mechanically transmissible (12, 133), but accuracy is affected by the virus source as well as the concentration and extraction methods (133). Christoff (36) and Kegler (106) attempted to determine thermal inactivation points for certain tree fruit viruses by heating budwood in water and air for the prescribed 10-min periods and then budding to healthy seedlings. Data were obtained by indexing the seedling or the explant if they grew. The difficulty with *in vivo* studies is that viruses may have thermal inactivation points higher than the thermal-death-point of the host plant.

Thermal inactivation of plant viruses *in vitro* follows the course of a first-order chemical reaction (2, 12, 157, 202). Noting this fact, Price (157) suggested that the velocity constant of inactivation at a specified temperature would better express temperature relationships of a virus than its thermal inactivation point, since this constant could be determined with greater accuracy from an equivalent amount of data. He further suggested that a true picture of the heat stability of the virus could be drawn if the velocity constants of inactivation were determined for a virus through a range of temperatures under specified conditions. Strangely, only a few kinetic studies have been made of the inactivation *in vitro* of plant viruses (2, 12, 157,

202) or plant virus nucleic acid (60) in line with Price's suggestion, and none has been made for the warm-air inactivation of plant viruses *in vivo*. On the other hand, kinetic studies with animal viruses (151, 205) have enabled the formulation of sophisticated theories on the mechanisms of thermal inactivation at the molecular level.

Although no specific study has been made of the inactivation of a plant virus *in vivo* through a temperature range, Fenrow et al. (46) plotted the thermal-death-curve of potato leafroll virus between 32 and 40° C from their own data and data from the literature on inactivation of this virus in tubers. The curve was a straight line when temperature was plotted against the logarithm of inactivation time. This indicates that the inactivation of potato leafroll virus *in vivo* proceeds as a first-order reaction even when inactivation times are determined under a wide array of environmental conditions.

An advantage is readily apparent from using velocity constants of inactivation for *in vivo* studies of viruses in perennial crops. One can show that half-life is an alternative way of expressing this constant; and with sufficient populations of infected test plants in the heat chamber the half-life at a specified temperature for any virus, whether or not mechanically transmissible to a local-lesion assay host, can be estimated by statistical methods (37). Thus the kinetics of the inactivation of any plant virus that is inactivated before its host plant dies from the treatments can be studied through a range of temperatures. For viruses that are cured in infected perennial plants, we suggest that half-life of the virus *in vivo* at 38° C is a more meaningful statistic than the *in vitro* thermal inactivation point. The half-life *in vivo* at 38° C expresses the thermal stability of the virus, complementing the half-life *in vitro*, which expresses longevity as suggested by Yarwood & Sylvester (208). The concept of Yarwood & Sylvester should be defined more precisely as half-life *in vitro* at 20° C since temperature affects any biological reaction rate (96).

Most published studies give only fragmentary consideration to the kinetics of heat tolerance of the host plant, possibly on account of the expense of more than a single heat chamber but more probably because the purpose in most studies has been the production of clean mother stocks rather than understanding of the principles of heat therapy. Straight-line survival curves, where determined (14, 158), indicate the host killing is a rate process (96) much like inactivation of the virus. Whether the temperature coefficient of inactivation of the virus is the same as that of its host is unknown for most viruses. Data of KenKnight (108) suggest that the thermal-death-curve of peach rosette virus and dormant *Prunus* buds in hot water might intersect at high temperatures. Kunkel (112) found that the difference between the minimum inactivation period for peach yellows virus and the maximum survival period of peach buds was greater at lower than at higher temperatures. This probably indicates the reason that most successful virus inactivations are carried out near 38° C, a temperature that is lethal to viruses

but that can be withstood for long periods with adequate light by a conditioned host, particularly a perennial.

There is no support for an early hypothesis that virus is not completely inactivated during heat treatments (101). Within the plant there may be centers where virus still remains even with relatively prolonged heat treatment, though the danger of such persistent infections is eliminated by propagating heated buds or tips. Clones of grapevines and fruit trees are still free of virus that were freed of virus by heat treatments at Davis 10 years ago and protected against reinfection in the field. In early work at Davis we were unable to free entire grapevine plants of fanleaf by heating at 38° C for 64 days, but when the tolerance of the host to heat was increased and the exposure extended to 102 days (64), the virus was eliminated completely. Many workers have had similar experience.

The hypothesis that heat therapy results from a shift in balance between virus synthesis and degradation (101, 104, 105) in which synthesis is reduced is probably true at temperatures sublethal for both the virus and the host. Here, titer may be the expression of balance between synthesis and degradation of virus. At higher temperatures, however, synthesis does not occur, and inactivation of the virus results from heat. The hypothesis does not account for the ultimate extinction of virus in the surviving host tissues after prolonged treatments at the critical temperature-time.

The most plausible hypothesis for the mechanism of heat therapy is that high temperatures cause the destruction of essential chemical activities in both virus and host but that the host is better able to recover from the damage. In other words, the temperature coefficient of thermal inactivation for the host exceeds that of the virus at certain temperatures, as suggested by Geard (57). This mechanism is probably not different from that of heat treatments used for killing other pathogens intimately associated with a host plant.

The inactivation process *in vivo* may be purely physical, as it seems to be *in vitro*, or it may be aided by biological changes induced in the host metabolism by high temperatures (56).

Campbell (27) speculated that therapy might result from the immobilization of virus, so that new tips on rapidly growing tissues in the chamber remain virus-free. Welsh & Nyland (203) found, however, that buds already formed and infected before heating were freed of virus by the heating. Mellor & Stace-Smith (132) and McGrew (122) found that the only obviously significant variables for heat-therapy success were temperature, length of treatment, and size of tips taken for starting explants; and that position or rate of growth in the chamber had no bearing on it. These findings suggest that heat therapy results from inactivation of virus, not immobilization.

Whether temperature-time treatments or the size of tips taken for starting explants is more important in plant-virus therapy will depend on the specific virus and its invasiveness within the host. Holmes (88) found that

tips from dahlia plants infected with spotted-wilt virus propagated free from the disease at normal growing temperatures. On the other hand, Stace-Smith & Mellor (178) found that axillary bud tips 1 to 3 mm long from potato plants infected with PVX were generally free of the virus only after 26 weeks at 35° C, whereas a few tips less than 500 μ long were free without heating. Whether any virus is completely systemic within its host plant is not established. Sheffield (173) detected tobacco mosaic virus in the apices of roots and stems of tomato plants less than 100 μ long, and Walkey & Webb (200), in electron-microscope studies, observed virus particles and tubules enclosing particles in the meristem dome of *Nicotiana rustica L.* infected with cherry leafroll virus. Many workers (10, 135, 160), however, have obtained clean plants through cultures of meristems from virus-infected mother plants.

Mathews & Lyttleton (129) found that turnip yellow mosaic virus in plants held at 35° C lost infectivity without significant changes in other virus properties. Babos & Kassanis (2), in *in vitro* studies, observed that inactivation of tobacco necrosis virus (TNV) proceeded exponentially at different temperatures, as if it were a two-component system with rapidly inactivating and slowly inactivating fractions. Nucleic acid extracts of TNV inactivated similarly to the intact virus at 51° C, indicating that inactivation resulted from changes in virus nucleic acid. Those workers also found inactivation to occur without significant changes in other virus properties or virus morphology. Additional kinetic studies of viruses *in vivo* as well as *in vitro* should shed further light on the mechanisms of successful heat therapy of plant viruses.

Uses of Heat Therapy

Heat therapy has both basic and practical applications. It is a tool both in identifying viruses and in separating them from disease complexes. Practically, it is indispensable to producing clean nuclear stocks where clonally propagated crop plants have become completely infected with one or more viruses. It has been used both deliberately and unintentionally as a direct treatment for controlling different plant virus diseases under field conditions.

The identification of viruses or virus relationships is impossible for many viruses of perennial plants because the viruses cannot be isolated from the diseased host. Thus, properties such as morphology and serology which might demonstrate relationships or lack of relationship of the etiologic agents cannot be studied directly. A very useful tool for indicating relationships among such viruses could be the half-life *in vivo* at 38° C. Heat treatment might succeed where serological methods fail (155).

Heat inactivation *in vivo* can help identify individual viruses where complexes exist (152, 203), or it might even establish that a specific disease is caused by a virus (53).

Heat therapy has aided understanding of some problems of rootstock-

scion incompatibilities and might be useful in studying others. Stubbs (187) found that Josephine pear budded onto an apple rootstock grew vigorously when held in a heat chamber for 2 to 3 months at *ca* 38° C, whereas without heat the graft combination failed.

Pure cultures of heat-stable viruses from virus complexes are possible through differential heat inactivation in cases where virus vectors or differential host plants are unknown (45, 146). Heat treatments are particularly useful for separating labile from stable components in raspberry virus studies (34, 37, 176). Hildebrand (75) found that the symptoms of sweet potato russet crack were masked until heat therapy eliminated the more labile internal cork virus.

The chief practical achievements of heat therapy are clean planting stock in a few cases, and mother plants free of virus disease. Sugarcane viruses (chlorotic streak and ratoon stunting) are controlled by hot-water treatments of the cane cuttings (setts). Annually, several thousand tons are treated in large tanks in hot water or in hot-air ovens, where accurate control of temperature permits virus eradication without appreciable damage to the setts themselves (68, 104, 181).

After Kassanis (100) eradicated leafroll from potatoes it seemed that heat treatments of seed tubers might be used effectively in potato production. Such treatments, however, caused too much damage to the tubers for direct planting (71, 165); and Dutch workers (166, 197) reported that the treatments caused mutations to develop in the new plants from the heated tubers. The symptoms that they observed, however, appear to have been temporary growth responses resulting from partial inactivation or reinvasion by other potato viruses in the young plants. No further reports of plant mutations resulting from heat treatments are known. Perhaps even the effect of tuber damage is overestimated as recent work from Czechoslovakia (175) indicates that heat treatments, especially when applied to tubers in the fall, reduced the incidence of leafroll and increased potato yields. In any event heat treatments are useful for developing sources of nuclear potato stocks (165).

Carnation growers are using heat therapy directly. In south France, carnation stocks are produced in greenhouses where temperatures are held at 40° C for 1 month before tip cuttings are taken for propagation (161). The production system also involves the indexing of cuttings to *Chenopodium amaranticolor* Coste & Reyn. and should prove very effective for the suppression of certain carnation virus diseases if standards for indexing are sufficiently high.

Heat treatments have been employed most widely for ensuring virus freedom in mother plants used for the production of foundation stocks. Here, heat therapy is followed with thorough indexing over a period of 1 to 3 years before the plants are considered free of known viruses. They are then grown in isolated plantings or screenhouses as source plants for clean planting stocks. This procedure has been very successful in California for

the production of clean stocks of grapevines and stone fruits. Disease-free stock produced for spring planting in 1969 included over 2,000,000 grapevine cuttings and almost 100,000 fruit trees. Advantages from stocks originating from heat-treated mother plants can be cited for several crops: apples (30), carnations (85, 149), chrysanthemums (78), peaches (124), pears (29), and strawberries (5), to list a few.

One's point of view sometimes determines whether freedom from disease is beneficial or not. Certain cactus fanciers measure their success by the number of species or forms of cacti that they have in their collections. Nozeran & Neville (142) noted that such fanciers inoculated their plants with *Opuntia* witches' broom virus to create additional monstrosities. This virus is very easily inactivated by heat, and one might suppose that in this isolated case heat therapy would be deleterious if it reduced the fancier's collection to half its original number. Some nurserymen look with trepidation on our current investigations on heat inactivation of camellia variegation virus, since it might lessen the demand for all variegated forms. In some cases, inactivation of mild strains of some viruses might not be desirable.

Heat need not be applied in sophisticated heat chambers for the successful eradication of plant viruses. Sometimes, in fact, reduction of natural heat can favor disease. Thus, potato leafroll was unknown on the plains of the river valleys of India when seed tubers were stored in thatched huts for 6 months during the hot summer season, but it became a very serious problem when cold-storage facilities were built or when seed tubers were brought in from the cooler mountain districts (191). Frazier et al. (48) found that natural heat eliminated certain strawberry viruses if plants were grown in an area with high summer temperatures, and observations on the effects of high summer temperatures on peach yellows started Kunkel's scientific investigations of the possibility of heat therapy for controlling virus disease.

One can take advantage of natural heating to control certain virus diseases. Thus, Fulton (51) set the minimum temperature in a plastic chamber in his greenhouse at 31° C, and natural heating eliminated a virus complex from strawberry plants growing in the chamber. In South Africa, McClean (121) built polyethylene tents over citrus trees infected with greening virus and found that on hot clear days the maximum temperature within the tent could go to 45 to 54° C. When the temperature exceeded 43° C under these tents, trees exposed for periods of 3 days to a week survived and showed considerable remission of greening symptoms in comparison with uncovered adjacent checks.

Heat treatment is being utilized in California to assist in moving plant material from areas quarantined for certain virus diseases. Heat treatment assures freedom from tomato ringspot in stone fruits and is substituted for a 3 year index on indicator plants. Several accessions of plants introduced into the U.S.A. from other countries have been made available

through heat treatment when they would otherwise have been excluded. Heat treatment should be adopted as standard procedure for the movement of plants through quarantines.

In the 1890's, careful clean-stock programs were put into effect in Java to control the sereh disease of sugarcane (204). Only clean stocks grown in certain mountain districts were used for planting new fields. The success of these programs can be deduced from the fact that the sereh disease no longer exists (43). Kobus and his associates and Wilbrink (204) were able to inactivate the etiologic agent with hot-water treatments, and it is on this basis that we assume that it was caused by a virus. Peach yellows in the eastern U.S.A. has reached the near-vanishing point because of tree removal and other control measures. Complete eradication may not be necessary; the disease may disappear when the incidence falls below a survival threshold. Clean-stock programs based on heat therapy and indexing should relegate many other plant virus diseases to the limbo of sugarcane sereh and peach yellows.

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