Thermotherapy of Russet Burbank Potato Tubers and Plants Infected with Alfalfa Mosaic Virus

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ABSTRACT

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Alfalfa mosaic virus (AMV) was eradicated from tubers of greenhouse- and field-grown Russet Burbank potato by hot-air treatment at 37 C for 4–5 wk. Virus could not be detected by repeated inoculation of susceptible test plants or by gel immunodiffusion tests in plants arising from these heat-treated tubers. Heat treatment effectively eliminated AMV from all sizes of tubers tested (<50–>100 g). Survival of heat-treated tubers after 4 and 5 wk was ≥60% regardless of tuber size. The virus also was eliminated from shoot tips (1–1.5 cm) of AMV-infected plants after 6–8 wk of exposure to alternating temperature regimes (35–37 and 27 C). Exposure of whole virus-infected plants to such treatment for 6–14 wk caused temporary remission of virus symptoms; however, plants were still infected with AMV after removal from the elevated temperatures. Continuous exposure of AMV-infected plants at 35 C resulted in death of all heat-treated plants within 6 wk, before AMV-free shoot tips could be propagated.

Additional key words: potato virus

Numerous viruses are reported to infect potatoes worldwide (2,3). Most, if not all, potato viruses can be transmitted in vegetatively propagated tubers. Important sources of virus frequently are seed tubers used to plant commercial fields. In a recent study, Thomas (8) found that several potato viruses, including S, X, Y, and leafroll, were present in varying amounts in tubers used as seed in Washington's Columbia Basin.

New or improved techniques that effectively eliminate virus infection in potatoes are highly desirable for plant quarantine and seed certification purposes. These could be used in conjunction with other seed certification procedures to produce healthier, higher-quality seed potatoes.

Since the pioneering work of Kassanis (5) in 1949, researchers worldwide have used heat to free potato tubers of potato leafroll virus (PLRV). In 1980, Kaiser (4) demonstrated the efficacy of thermotherapy to free potato tubers of alfalfa mosaic (AMV), potato leafroll (PLRV), and tomato black ring (TBRV) viruses. This study describes the use of hot-air treatment to free Russet Burbank shoot-tip cuttings and tubers of AMV.

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MATERIALS AND METHODS

Source of virus. All tests were carried out with one isolate of AMV from a naturally infected Russet Burbank potato plant collected at Othello, WA. The virus was selected after serial single-lesion transfer on *Vigna unguiculata* 'California Blackeye' cowpea. This particular isolate of AMV also produced systemic yellow mosaic symptoms in California Blackeye cowpea. The virus was maintained in infected dormant Russet Burbank tubers at 4–6 C or in infected potato plants in the greenhouse at 15–25 C.

Source of potato plants and tubers. All Russet Burbank potato plants and tubers used in this study were derived from three PVX-tested tubers from an elite seed potato source from Manhattan, MT. The three tubers were sprouted and planted in sterile potting medium (55% peat moss, 35% pumice, 10% sand) in the greenhouse, where temperatures ranged from 15 to 25 C. Each parent plant was assayed for virus infection by host range, serology, electron microscopy, and vector transmission studies. The three parent plants were found to be free of potato viruses A, M, S, X, Y, and potato leafroll and other viruses, such as AMV and cucumber mosaic. When the parent plants were 10–15 cm tall, they were inoculated with the potato AMV isolate. Once it was established that the three parent plants were infected with AMV (by back-inoculation to California Blackeye cowpea and serology), stem cuttings (3–5 cm long) were taken from each plant, treated with a rooting hormone (Rootone F), and planted in moist vermiculite. Rooted cuttings were transplanted to sterile potting medium in 15- or 25-cm-diameter plastic pots.

Tubers used in the thermotherapy experiments were obtained from AMV-infected plants grown in the greenhouse or field. This was done to determine whether the cultural and environmental conditions under which virus-infected plants were grown had any effect on subsequent survival and eradication of virus from these tubers when exposed to 37 C for periods up to 5 wk. Greenhouse-grown tubers were collected from AMV-infected plants that were grown in an elevated ground bed where the soil had been fumigated with methyl bromide. Field-grown tubers were obtained from AMV-infected plants that had been transplanted to a field at an isolated location near Central Ferry, WA. The soil around each plant was treated with aldicarb (Temik). Plants in the greenhouse and field were sprayed periodically with pesticides to control insects and mites. After harvest, tubers were stored at 4–6 C for 2 mo before being used in heat-treatment experiments.

Hot-air treatment of tubers. Tubers from field- and greenhouse-grown plants were divided into three groups according to weight: >100, 50–100, and <50 g. Tubers from each weight class were placed in paper bags, which were placed in a dark growth chamber at 37 ± 0.5 C. The relative humidity (RH) in the growth chamber was maintained at >75% by filling large shallow trays with water. Unheated control tubers were maintained at room temperature (20–25 C) in paper bags until planted. Tubers were removed from the growth chamber after 3, 4, and 5 wk and sprouted at room temperature. Sprouted tubers were planted in sterile potting medium in 8-cm-diameter plastic pots and placed in the greenhouse. When plants were 6–8 cm tall, they were indexed for AMV. Leaves were triturated in 0.06 M K_2HPO_4 and the sap was applied to young leaves of California Blackeye cowpea dusted with 0.22-μm (600-mesh) Carborundum. Plants found to be free of AMV were retested for virus infection two or three times during the first season of growth. Tubers from selected plants that were negative for AMV were sprouted and reindexed at least twice during the second growing season. Additionally, randomly selected plants were tested for AMV infection by agar double-diffusion tests.

Hot-air treatment of plants. Eight-week-old AMV-infected potato plants were clipped to about 20-cm height to stimulate new growth and placed in growth chambers. Plants were subjected to four periods of continuous or alternating high and low temperature regimes with a 16-hr photoperiod (11,500 lux) and >75% RH. Temperature regimes were: 27/20 C = 27 C (16 hr) + 20 C (8 hr); 35 C = 35 C (24 hr); 35/27 C = 35 C (10 hr) + 27 C (2 hr) + 35 C (10 hr) + 27 C (2 hr); and 37/27 C = 37 C (8 hr) + 27 C (4 hr) + 37 C (8 hr) + 27 C (4 hr). The temperature in each growth chamber was increased gradually over a period of 7–10 days; when the specified temperatures were reached, the treatment period started (day 1). At biweekly intervals, shoot-tip cuttings (1–1.5 cm) were taken with a sterile scalpel from each surviving plant, dusted with Rootone F, and planted in moist vermiculite in 8-cm-diameter plastic pots, which were placed in saucers of water and covered with a glass plate (to maintain a high RH). Rooted tip cuttings were transplanted to sterile potting medium in the greenhouse. When plants were 6–10 cm tall, they were assayed for AMV infection on California Blackeye cowpea. Plants indexing negative for AMV infection were reindexed three or four times during the growing season. Tubers from these AMV-free plants were sprouted and indexed again for AMV on California Blackeye cowpea test plants and by immunodiffusion tests.

RESULTS

Inactivation of AMV from greenhouse-grown tubers. Virus was not detected in any surviving tubers exposed to hot air at 37 C for 4 and 5 wk (Table 1). Inactivation of AMV occurred in tubers regardless of weight. Survival of tubers in the three weight classes ranged from 80 to 100%. The curative effect of heat treatment was observed after 3 wk, when inactivation of AMV from tubers ranged from 20 to 67%. In unheated tubers (control), 97% of the tubers were infected with AMV and survival of tubers was >93%.

The potato isolate of AMV used in this study produced conspicuous yellow mosaic (calico) symptoms in plants arising from virus-infected tubers in the control and 3-wk treatments. Plants that assayed negative for AMV after heat treatment for 3, 4, and 5 wk were dark green and never showed calico symptoms. Virus (when repeatedly assayed by rub-inoculation of the juice to cowpea test plants and serology) was not detected in first-generation plants of heat-treated tubers in the 4- and 5-wk treatments. Indexing techniques have proven consistent in detecting AMV in virus-infected potatoes; thus, the results presented are considered reliable.

Inactivation of AMV from field-grown tubers. More tubers in the size classes of 50–100 and >100 g were obtained for testing from the AMV-infected potato planting at Central Ferry than from plants grown under greenhouse conditions at Pullman, WA. Ninety-five to 100% of the field-grown control tubers in the three weight classes were infected with AMV (Table 1). Effects of the hot-air treatment could be observed after 3 wk, when 17–25% of the tubers were virus-free. At 4 wk, the number of AMV-free plants increased from 29 to 76% for tubers weighing >100 and <50 g, respectively. Survival of tubers at 3 and 4 wk was 75% or higher. All tubers that sprouted in the 5-wk treatment series were free of AMV. Survival of heat-treated tubers at 5 wk was 60-70%. Plants from the different size classes of tubers infected with AMV usually showed calico symptoms, particularly in the early stages of plant growth. AMV was not detected in first- and second-generation tubers from selected virus-free plants exposed to hot air at 37 C for 3, 4, or 5 wk.

Inactivation of AMV in shoot tips. After several weeks in the growth chamber at continuously elevated temperatures, plants became spindly and their rate of growth decreased markedly. Heat treatment resulted in a temporary remission of calico symptoms in all surviving plants. Survival of potato plants receiving the 37/27 C treatment was superior to that of the other elevated-temperature regimes tested (Table 2). Fifty percent of the plants survived 14-wk treatment at 37/27 C. AMV-free cuttings were obtained from plants exposed to alternating temperatures of 37/27 C after 8 wk, when one of 52 rooted cuttings contained no detectable AMV. At 14 wk, four of 10 rooted shoot tips from three plants incubated at 37/27 C indexed free of AMV. When the three mother plants (nos. 20, 21, and 22) were assayed for AMV 3 wk after removal from the growth chamber (after 14 wk), all were still infected with the virus. Virus-free shoot tips were first obtained from two plants that were exposed to alternating temperatures (37/27 C) for 6 wk (Table 2). One plant (no. 9) survived 14 wk. Plant 9 continued producing at least one virus-free cutting on each of the successive biweekly sampling dates and produced three virus-free cuttings at 14 wk. This plant was still infected with AMV, however, when new foliage was indexed for virus 69 days after removal from the heat chamber. Within 2 wk, most plants at a continuous 35 C started turning chlorotic and dropping their leaves, and by 4 wk, 50% were dead (Table 2). All plants at 35 C were dead

Table 1. Effect of heat treatment (37 C) duration and weight of greenhouse and field-grown Russet Burbank potato tubers on inactivation of alfalfa mosaic virus[a,b]

Treatment period (wk)	Tuber weight (g)	Survival of tubers		Surviving tubers			
				Greenhouse		Field	
		Greenhouse	Field	Infected (%)	Healthy (%)	Infected (%)	Healthy (%)
0	>100	14/15[c]	20/20	100	0	100	0
	50–100	15/16	20/20	100	0	100	0
	<50	33/33	19/20	97	3	95	5
3	>100	5/5	15/20	80	20	80	20
	50–100	15/15	15/20	40	60	73	17
	<50	47/55	16/20	33	67	75	25
4	>100	4/5	17/20	0	100	71	29
	50–100	15/16	18/20	0	100	50	50
	<50	46/55	17/20	0	100	24	76
5	>100	4/4	12/20	0	100	0	100
	50–100	15/17	14/20	0	100	0	100
	<50	48/55	13/20	0	100	0	100

[a] Unsprouted tubers were incubated in a growth chamber at 37 ± 0.5 C in the dark at a relative humidity of >75%.
[b] Tubers were harvested from AMV-infected potato plants that were grown in an elevated soil bed in the greenhouse at Pullman, WA, or in the field at Central Ferry, WA.
[c] Number of tubers surviving treatment divided by total number treated.

within 6 wk. None of the 14 shoot-tip cuttings made at this temperature after 4 wk were free of AMV.

Several tubers were collected from each of the 20 plants derived from AMV-free shoot-tip cuttings from plants treated at 35/27 or 37/27 C. These were sprouted and assayed for virus on California Blackeye cowpea. None of the plants arising from 42 sprouted tubers contained detectable AMV. Conversely, all first-generation plants from 25 tubers of control plants treated at 27/20 C were virus-infected.

DISCUSSION

Results of these thermotherapy experiments agree with those of previous work (4) in which AMV was eradicated from 100% of the tubers of two potato cultivars within 4–5 wk. Survival of tubers at 4 wk was similar whether grown in the greenhouse or the field (80–94%), but at 5 wk, it was less (65%) in field-grown than in greenhouse-grown (88%) tubers. This difference could reflect damage or other physiological and/or chemical changes that occurred in field-grown tubers during growth, harvest, or storage, which reduced their survival in hot air for periods longer than 4 wk.

Commercial growers establish new potato plantings with whole or cut seed tubers. The desired weight of seed pieces ranges from 43 to 57 g (1.5–2.0 oz) (10). In this study, hot-air treatment at 37 C for 4 or 5 wk eliminated AMV from tubers in all weight classes tested (<50–>100 g). This appears to be the first time thermotherapy has been used successfully with large tubers (>100 g). In an earlier study (4), it was shown that AMV could be eliminated from tubers of two potato cultivars that ranged in weight from 0.5 to 10 g. The present study suggests that size affects the time required to free tubers of AMV. At 3 wk with greenhouse-grown tubers and 4 wk with field-grown tubers, AMV was eliminated from a higher percentage of the tubers in the smallest weight class (67–76%) than from those in the largest class (20–29%). Inactivation of AMV may proceed at a slower rate as tuber size and volume increase, particularly if temperatures near the center of the tuber are lower than those nearer the surface.

Research on eradication of AMV from plant tissues by heat has been limited. Frosheiser (1) was the first to demonstrate the efficacy of thermotherapy and shoot-tip culture to free alfalfa (*Medicago sativa* L.) plants of AMV. He eradicated AMV from shoot tips (1–2 cm) of several alfalfa lines exposed to hot air (35–36 C) for 8–80 days. Walkey and Cooper (11) eradicated AMV from infected *Nicotiana rustica* L. meristem tips grown in shake culture at 32 C for 98–120 days.

Excision of shoot tips (<1.0 cm) from plants exposed to hot air at 33–38 C produced virus-free stocks of different potato cultivars (6,7,9). Mellor and Stace-Smith (6,7) eradicated potato virus X, but not S, from shoot tips (0.1–0.6 cm) of Russet Burbank plants incubated at air temperatures of 33–37 C for periods up to 29 wk. A treatment period of 15 wk was required before the first PVX-free shoot tip was obtained from heat-treated plants (6,7). Both PVX and PVS appear to be more thermostable than AMV because AMV-free Russet Burbank shoot tips (1–1.5 cm) were obtained after 6 wk at alternating temperatures of 27–35 C.

The heat tolerance of Russet Burbank plants exposed to continuous (35 C) or alternating (27–37 C) temperatures proved to be a critical factor for survival of AMV-infected plants and eradication of virus from excised shoot tips. Plants exposed to a continuous air temperature of 35 C died before AMV was eradicated from shoot tips, whereas alternating periods of high (35–37 C) and low (27 C) temperatures resulted in prolonged survival of heat-treated plants and eradication of virus from shoot tips within 6–8 wk. Mellor and Stace-Smith (6) also observed that longer treatment periods at air temperatures of 33–37 C were more effective in eradicating PVX from shoot-tip cuttings than an average air temperature of 37 C.

Table 2. Inactivation of alfalfa mosaic virus in shoot-tip cuttings of Russet Burbank potato plants incubated at different temperatures for various time intervals[a]

Temperature[b] (C)	Plant no.	Survival of shoot-tip cuttings at biweekly intervals					
		4	6	8	10	12	14
27/20	1	0/7[c]	0/5	–[d]	0/2	0/2	0/6
	2	0/8	0/6	–	0/3	–	0/3
	3	0/7	0/6	–	0/1	–	0/2
	4	0/6	0/5	–	0/4	–	0/3
	5	0/6	0/2	–	0/6	–	0/6
	6	0/7	0/7	–	0/4	–	0/2
35/27	7	0/1	Dead[e]	...	...	...	...
	8	0/5	0/1	Dead	...	...	...
	9	0/2	1/2	1/3	1/7	1/4	3/3
	10	0/6	0/3	Dead	...	...	...
	11	0/6	1/6	1/1	Dead	...	...
	12	0/2	0/1	Dead	...	...	...
35	13	0/5	Dead	...	...	...	...
	14	Dead	...	...	...	...	...
	15	Dead	...	...	...	...	...
	16	0/7	Dead	...	...	...	...
	17	Dead	...	...	...	...	...
	18	0/2	Dead	...	...	...	...
37/27	19	0/8	0/7	–	Dead	...	...
	20	0/8	0/8	0/12	0/6	1/2	1/1
	21	0/8	0/8	0/15	0/6	2/8	2/7
	22	0/8	0/8	0/12	1/7	1/1	1/2
	23	0/8	0/8	1/5	1/1	Dead	...
	24	0/8	0/8	0/8	Dead	...	...

[a] Plants were incubated in a growth chamber for different periods of alternating high and low temperatures at a 16-hr photoperiod (11,500 lux) and relative humidity of >75%.

[b] Temperatures were 27/20 = 27 C (16 hr) + 20 C (8 hr); 35/27 = 35 C (10 hr) + 27 C (2 hr) + 35 C (10 hr) + 27 C (2 hr); 35 = 35 C (24 hr); and 37/27 = 37 C (8 hr) + 27 C (4 hr) + 37 C (8 hr) + 27 C (4 hr).

[c] Number of rooted shoot-tip cuttings (1–1.5 cm) free of AMV divided by the total number of rooted cuttings tested for virus infection.

[d] – = Plants still alive, but shoot-tip cuttings not taken.

[e] No shoot-tip cuttings taken because plant had died.

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