Preconditioning Grapefruit Callus Tissue Reduces Methyl Bromide-induced K⁺ Leakage

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Abstract. Callus tissue grown from 'Marsh' grapefruit (*Citrus paradisi* Macf.) albedo tissue was grown at 30C for ≈ 40 days. Calli were preconditioned in normal air for 5 days at 10 or 30C before being fumigated for 2 hr with 0, 32, or 48 g of methyl bromide (MB)/m³. Calli were then held at 10C and K⁺ leakage was measured after 1, 10, 20, and 30 days. The amount of K⁺ leaked from MB-fumigated calli was greater than that for nonfumigated calli and increased with higher MB dose. Leakage also increased with time following fumigation. Leakage of calli preconditioned at 30C and fumigated with 48 g MB/m³ was 140%, 196%, and 260% greater than leakage from nonfumigated calli 10, 20, and 30 days after fumigation, respectively. Leakage from calli preconditioned at 10C for 5 days before MB fumigation was less than that from calli held at 30C. MB doses of 32 and 48 g·m⁻³ increased leakage of calli preconditioned at 10C by 6% and 43% and for those preconditioned at 30C by 99% and 140%, respectively, 10 days after fumigation. In addition to K⁺ leakage, MB induced the development of a tan to orangish-brown discoloration.

Methyl bromide (MB), one of the few chemical fumigants still available for use on fresh citrus, is very effective in killing insects and leaves very little residue on the fruit. Fumigation of citrus with MB has been reported to control quarantined insects such as tephritid fruit flies (Benschoter, 1979, 1982; Williamson et al., 1986) and Fuller rose beetle (E. Soderstrom, personal communication).

Fumigation with MB, however, may cause a phytotoxic response in grapefruit and other citrus cultivars. The injury is characterized by a scald-like appearance on the peel that later may become water soaked (Hatton and Cubbedge, 1979). Injury caused by fumigation with MB is slow to appear and may take 3 to 5 weeks to develop (Armitage and Steinweden, 1946); it also may enhance decay (Hatton and Cubbedge, 1979; Benschoter, 1979).

The margin between MB doses needed for disinfestation and those causing phytotoxicity is narrow and the sensitivity of citrus fruit is variable (Williamson et al., 1986). From 0% to 92% of Florida grapefruit harvested at various times throughout the season developed injury from fumigation with MB at 40 g·m⁻³ for 2 hr (Hatton and Cubbedge, 1979). Lindgren and Sinclair (1951) suggested that the variable response of citrus to MB may depend on factors such as cultivar tolerance, fruit maturity, and the district where the fruit was grown. However, the physiological mechanism associated with MB in-

jury or tolerance is not known.

An in vitro system would facilitate the study of the physiological mechanism(s) that determines MB sensitivity and identification of factors that alter the sensitivity of the fruit. A system using callus tissue grown from grapefruit peel has been developed to study factors affecting chilling sensitivity (Forney and Peterson, 1990). Using this system, chilling sensitivity was reduced by preconditioning calli at 10 to 15C for 5 days before chilling. The objectives of the current study were to determine if 1) MB-induced injury could be studied using the grapefruit callus system and 2) preconditioning temperature influences the development of MB injury.

'Marsh' grapefruit were obtained from a tree grown at the Horticultural Crops Research Laboratory, Fresno, Calif. Grapefruit were washed and immersed in a 0.5% sodium hypochlorite solution for 20 min. The albedo tissue was aseptically cut from the fruit, and 50-mg cubes were cultured in 25 × 150-mm culture tubes containing 20 ml

of medium that contained Murashige and Skoog mineral salts, 3 μM 2,4-D, 1 μM kinetin, and (all/liter) 10 mg myoinositol, 2 mg glycine, 50 g sucrose, 10 g Bacto agar, and 100 ml orange juice that had been centrifuged 10 min at 10,000 × g to remove the pulp (Murashige and Tucker, 1969). Medium pH was adjusted to 5.7 using NaOH before autoclaving 15 min at 121C. Culture tubes containing explants were held at 30C for ≈40 days, during which time callus growth reached ≈1 to 1.5 g fresh weight. Calli were then preconditioned at 10 or 30C for 5 days. Calli that had been preconditioned at either temperature were placed into 28-liter fumigation chambers at 15C and allowed to equilibrate for 1 hr. Chambers were dosed with 0, 32, or 48 g MB/m³ and calli were exposed for 2 hr, followed by aeration. Calli were then held at 10C for up to 30 days.

The rate of K⁺ leakage was used as a measure of MB-induced injury (Forney and Peterson, 1990). Leakage rates were determined 1, 10, 20, and 30 days after fumigation on four calli from each combination of pretemperature and MB dose. Callus was removed from culture, cleansed of excess medium, and weighed. It was then put into a 125-ml Erlenmeyer flask containing 50 ml of distilled water and placed in a shaking water bath at 20C. After 2 hr, the tissue was removed from the water and frozen. The frozen tissue was leached for ≈5 hr in 100 ml of distilled water at room temperature. Potassium content of the leachates was determined electrochemically with a K electrode. The percent of total K⁺ content leached in 2 hr was calculated and used as a measure of injury. The experiment was conducted three times. The means of each experiment were analyzed using a randomized complete-block design, weighting means with the inverse of their associated variance.

In the third experiment, discoloration of calli was rated subjectively on a scale of 1 to 4, with 1 = no discoloration and 4 = severe discoloration. Calli were rated 1, 3, 7, and 14 days after fumigation.

Fumigation with MB caused an increase in the rate of K⁺ leakage from callus tissue held 10 or more days (Table 1). Leakage rates of fumigated calli were as much as 260% greater than nonfumigated controls. Rates of

Table 1. Percent of total K⁺ leached from grapefruit calli in 2hr as influenced by methyl bromide dose, preconditioning temperature, and holding time following fumigation.

Methyl bromide dose (g·m ⁻³)	Preconditioning temperature (°C)	% K ⁺ leached			
		Days held at 10C			
		1	10	20	30
0	10	28	21	22	22
	30	22	19	22	20
32	10	26	22	25	27
	30	33	37	56	63
48	10	33	30	43	52
	30	28	45	66	73
Significance					
Preconditioning temperature		NS	*	*	NS
Methyl bromide dose		NS	**	**	*

NS, *, ** Nonsignificant or significant at $P = 0.05$ or 0.01 , respectively, according to F test.

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Table 2. Discoloration rating of grapefruit calli as influenced by methyl bromide dose, preconditioning temperature, and holding time following fumigation.

Methyl bromide dose (g·m ⁻³)	Preconditioning temperature (°C)	Discoloration rating ^z			
		Days held at 10C			
		1	3	7	14
0	10	1.3	1.3	1.3	1.6
	30	1.1	1.1	1.3	1.0
32	10	2.0	2.3	2.9	3.9
	30	2.0	2.4	2.8	3.0
48	10	2.0	2.0	2.9	3.0
	30	2.0	2.4	2.9	3.0
Significance					
Preconditioning temperature		NS	NS	NS	**
Methyl bromide dose		**	**	**	**

^zDiscoloration rating: 1 = none, 4 = severe.

NS, **Nonsignificant or significant at $P = 0.01$, respectively, according to F test.

K⁺ leakage were greater from calli fumigated with 48 than from those fumigated with 32 g MB/m³. Similarly, in whole grapefruit, MB concentrations of 40 g·m⁻³ and higher caused injury symptoms to develop (Armitage and Steinweden, 1946; Benschoter, 1979; Hatton and Cubbedge, 1979).

The rate of K⁺ leakage from fumigated calli increased with time following fumigation (Table 1). One day after fumigation, leakage rates of treated calli were not significantly greater than those of controls. Leakage rates increased over time, with the calli preconditioned at 30C and fumigated with 48 g MB/m³ having 140%, 196%, and 260% higher rates than control calli 10, 20, and 30 days after fumigation, respectively. The development of injury on whole fruit was reported to be similar. Armitage and Steinweden (1946) observed no injury to grapefruit 5 days following fumigation, but began to observe injury after 9 to 14 days. Benschoter (1979) found that decay increased when grapefruit fumigated with MB were held at 10C for 4 weeks; however, he observed no increase in decay or peel injury when similarly treated fruit were held at 21 to 27C.

From these results, it appears the rate of K⁺ leakage was a good measurement of MB-induced injury in callus tissue. Increases in the rate of electrolyte leakage have also been shown to correlate with injury induced by chilling in grapefruit callus (Forney and Peterson, 1990), heat in muskmelon (Lester et al., 1988), and tomato (Inaba and Crandall, 1988), and exposure to ethylene in watermelon (Elkashif and Huber, 1988). Leakiness induced by MB may cause the increase in decay susceptibility of fumigated grapefruit observed by Hatton and Cubbedge (1979) and Benschoter (1979).

When calli were preconditioned at 10C for 5 days before MB fumigation, K⁺ leakage was 6% and 43% greater than for nonfumigated calli 10 days after exposure to 32 and 48 g MB/m³, respectively (Table 1). Leakage in calli held at 30C, however, was 99%

and 140% higher than in controls, respectively, indicating that low-temperature preconditioning increased the tolerance of calli to MB. After 20 and 30 days, differences between the 10 and 30C preconditioning treatment were even greater. Houck and Jenner (1988) reported a similar reduction in MB-induced injury when grapefruit, lemons, oranges, and tangelos were preconditioned at 15C for 1 week before fumigation. Low temperature preconditioning also increased the tolerance of grapefruit to chilling in callus (Forney and Peterson, 1990) and whole fruit (Hatton and Cubbedge, 1982).

In addition to increasing rates of K⁺ leakage, MB caused the development of discoloration in grapefruit callus tissue (Table 2). Discoloration of the creamy-white calli ranged from a light tan to an orangish-brown. Development of discoloration due to MB fumigation developed rapidly and was apparent after 1 day (Table 2). Severity of discoloration increased during the week following fumigation in both the 32 and 48 g·m⁻³ treatments. Unlike K⁺ leakage, severity of discoloration in calli fumigated with 48 g·m⁻³ was not greater than that of calli fumigated with 32 g·m⁻³. In fact, after 14 days, discoloration of calli treated with 32 g·m⁻³ was more severe than that in those treated with 48 g MB/m³.

Preconditioning calli at 10C did not affect the degree of discoloration for the first week following MB fumigation (Table 2). After 14 days, however, discoloration in calli treated with 32 g·m⁻³ was more severe in tissues preconditioned at 10 than at 30C.

Greater discoloration was observed in treatments that resulted in lower rates of K⁺ leakage. High rates of K⁺ leakage may indicate severe cell injury and/or cell death. If the discoloration observed in callus tissue is a result of altered metabolism induced by MB, dead or severely injured cells may no longer be capable of producing these pigments. In whole grapefruit, decay was shown to increase with increased doses of MB, but the effect of MB dose on peel injury was

variable (Benschoter, 1979). Increases in leakiness that may increase the susceptibility of fruit to decay appear to be a better measure of MB injury than discoloration.

Grapefruit callus tissue provides a convenient experimental system to study the physiology of MB sensitivity. Through preconditioning, MB sensitivity of calli can be altered in a predictable manner unlike the variable response of whole fruit. Determination of the mechanism(s) that affects MB sensitivity of callus tissue may lead to methods to reduce the damage caused by MB fumigation in whole fruit.

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