Low Temperature Storage Combined with Sulfur Dioxide Slow Release Pads for Quarantine Control of Omnivorous Leafroller *Platynota stultana* (Lepidoptera: Tortricidae)

VICTORIA Y. YOKOYAMA, GINA T. MILLER, AND CARLOS H. CRISOSTO[1]

Horticultural Crops Research Laboratory, USDA-ARS, 2021 South Peach Avenue, Fresno, CA 93727

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ABSTRACT Low temperature storage combined with sulfur dioxide slow release pads caused 100% mortality of omnivorous leafroller, *Platynota stultana* Walshingham, 2nd instar (the least susceptible stage to low temperature storage) after 3 wk of exposure in table grapes, *Vitis vinifera* L. Temperatures in packed grape clusters decreased from ambient to 2°C within ≈2 d after placement in storage. Sulfur dioxide concentrations in the grape clusters ranged between 0.2 and 1.0 ppm during the 1- to 6-wk storage period. The combination treatment incorporates existing packinghouse facilities and has potential to be used as an alternative to chemical fumigants such as methyl bromide to control pests of regulatory concern in exported table grapes.

KEY WORDS *Platynota stultana, Vitis vinifera,* quarantine treatments

OMNIVOROUS LEAFROLLER, *Platynota stultana* Walshingham, is found on grapes, *Vitis vinifera* L. (Flaherty et al. 1992), and has the potential to be a pest of regulatory concern on table grapes exported to countries where the insect is not found. Development of a postharvest treatment to control omnivorous leafroller in table grapes could help develop new export markets for fruit exported from the San Joaquin Valley of California.

Ahumada et al. (1996) developed insecticidal controlled atmospheres for 'Thompson Seedless' grapes that showed control of omnivorous leafroller in preliminary tests at 20°C for 4.5 d and at 5°C for 6 d. In related studies, low temperature storage was investigated as a potential quarantine treatment to control codling moth, *Cydia pomonella* (L.), oriental fruit moth, *Grapholita molesta* (Busck) at 0°C (Yokoyama and Miller 1989), and walnut husk fly, *Rhagoletis completa* Cresson, at 1–2°C (Yokoyama and Miller 1996) in exported stone fruits. However, low temperature alone did not attain complete control of these pests. The recommended storage and shipping temperature for table grapes is ≈0°C (Ryall and Harvey 1959). Our recent investigations with omnivorous leafroller showed that a low number of larvae survived 6-wk exposures to 0–1°C. The 2nd instar was the least susceptible stage when the eggs and 1st through 5th instars were tested at weekly durations of 1–6 wk (unpublished data).

Sulfur dioxide fumigation is used to control fungal infections and decay primarily caused by *Botrytis cinerea* Pers. on table grapes (Luvisi et al. 1992). Vail et al. (1992) reported the toxic effects of sulfur dioxide fumigation on omnivorous leafroller in acute exposures of 1,590–1,626 ppm for 0.2–1.0 h at 22°C. A sulfur dioxide generating pad has been developed to control botrytis in combination with a perforated polyethylene box liner to reduce water loss in California packed table grapes (Crisosto et al. 1994 1996). The sulfur dioxide generating pad has been used commercially for many years as a practical and economical method to control decay without injuring the fruit during extended storage and shipment periods.

The objective of this study was to determine the potential of low temperature storage combined with slow release sulfur dioxide generating pads as a potential combination quarantine treatment to control omnivorous leafroller in table grapes for export.

Materials and Methods

Insect Rearing. Omnivorous leafroller larvae were reared on a lima bean diet in the laboratory using methods developed for oriental fruit moth, *Grapholita molesta* (Busck) (Yokoyama et al. 1987). Pupae in cardboard strips and adults were transferred to ovipositional cages. The eggs were collected on waxed paper to produce larvae for rearing to the 2nd instar.

Preparation of Diet Cups with 2nd Instars. Diet (≈10 ml) was poured into small plastic cups (≈33 ml capacity, model P100, Solo, Urbana, IL). Eggs on waxed paper sheets were held in an incubator (27°C, photoperiod of 16:8, [L:D] h) until hatch (7 d). First instars were transferred into diet cups (7 per cup).

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[1] Kearney Agricultural Research Center, University of California, 9240 South Riverbend Avenue, Parlier, CA 93648.

The infested diet cups were placed in plastic cup holder trays (30 cups per tray, BioServ, Frenchtown, NJ) in an incubator (27°C, a photoperiod of 16:8, [L:D] h) for 4–5 d to obtain the 2nd instars. Three replicates of 12 trays (30 infested cups per tray) were used for controls to determine natural mortality without exposure to sulfur dioxide and low temperature.

Packing of Table Grapes and Infested Diet Cups in Foam Containers. Grape clusters of 'Ruby Seedless' were packaged as described by Luvisi et al. (1995) and Crisosto et al. (1994) in polyethylene cluster bags (≈1.36 kg per bag). Six cluster bags were placed in a foam (expanded polystyrene) container (outer dimensions, 40 wide by 50 long by 17 cm high) lined with a perforated polyethylene box liner described by Crisosto et al. (1996). Three diet cups containing 2nd instars were placed in each of the 6 cluster bags of grapes. Twelve infested diet cups were placed at random inside the foam container and box liner among the grape filled cluster bags. A total of 30 diet cups with 2nd instars (7 per cup) were used per foam container. Four foam containers were prepared in this manner to make a replicate. Three replicates were used per exposure period.

Monitoring Temperature and Relative Humidity. One temperature and 1 relative humidity datalogger (Stowaway, Intermountain Environmental, Logan, UT) were placed in a foam container packed with grapes and infested diet cups for each replicate. The sensor for each data logger was placed among the grapes in a cluster bag. The time (hours) for the air temperature among the grape clusters to drop to 2°C after placement in low temperature storage was reported as the mean ± SE. The highest and lowest temperatures in the packed foam containers after 2°C was attained was reported as the mean ± SE of the replicates.

Exposure to Sulfur Dioxide and Low Temperature Storage. A slow phase sulfur dioxide pad containing anhydrous sodium bisulfite (7 g) (20 wide by 38 cm long, 12 packets per pad) (Grape Guard, Uvas, Santiago, Chile) was placed on an absorbent paper liner (26 wide by 37 cm long) on top of the 6 clusters of grapes in polyethylene bags packed in each foam container. The box liner was folded and loosely sealed with cellophane packaging tape and the lid placed on the box. The container was placed in 0–1°C cold storage facilities at either the USDA-ARS, Fresno, CA, or the University of California Kearney Agricultural Research Center, Parlier, CA, The grapes and 2nd instars in diet cups were held in cold storage at exposures of either 1, 2, 3, 4, 5, or 6 wk.

Determination of Sulfur Dioxide Concentrations. A pump-type gas sampler (Luvisi et al. 1992) was used to determine sulfur dioxide concentrations. Samples were taken from each of 5 replicate containers selected at random at 1 and 3 d, and at weekly intervals for 6 wk after placement in cold storage. Polyethylene tubing (4 mm i.d. by 27 cm long) was placed in the center of the container among the grapes in a cluster bag. A sulfur dioxide low range detector tube (0.05–10 ppm) (Gastec, Ayase, Japan) was attached to the end of the tubing. Gas samples were drawn through the detector tube with a pump (Matheson-Kitagawa model 8014-400A, Matheson Gas Products, Montgomeryville, PA). Gas concentrations (ppm) were reported as the mean ± SE of the 5 replicates per time interval.

Table 1. Time to attain 2°C from ambient air temperature among grape clusters with omnivorous leafroller in diet cups, packed in foam containers after placement in low temperature storage, and high and low temperatures during each exposure period thereafter (mean ± SE of 3 replicates)

Storage, wk	Time to 2°C, h	High temp, °C	Low temp, °C
1	32.0 ± 16.9	2.1 ± 0.7	1.3 ± 0.4
2	1.0 ± 0.0	1.8 ± 0.8	1.1 ± 0.5
3	51.0 ± 13.2	1.5 ± 0.7	1.1 ± 0.6
4	28.0 ± 12.9	1.9 ± 0.7	1.1 ± 0.4
5	13.3 ± 10.9	1.2 ± 0.4	1.0 ± 0.4
6	47.0 ± 25.0	1.4 ± 0.5	1.2 ± 0.5

Evaluation of Insect Mortality. Second instars in diet cups were removed from the packed grapes after the end of the test storage period. The cups were returned to plastic trays and incubated (27°C, a photoperiod of 16:8, [L:D] h) with the controls for a minimum of 13 wk. Adults that emerged from the diet in the treated and control groups were considered survivors. The results were reported as the mean ± SE percentage mortality of 3 replicates for each low temperature storage period and the controls.

Results and Discussion

Cold storage facilities were set at an air temperature of 0–1°C. Temperatures decreased from ambient to 2°C within ≈2 d among the grape clusters with omnivorous leafroller 2nd instars in diet cups (Table 1). Thereafter, temperatures fluctuated between ≈1–2°C simulating normal packinghouse storage conditions. The period to reduce temperatures inside the foam container after placement in cold storage may have been affected by the packaging materials. Mean time to attain 2°C was 28.7 ± 7.8 (mean ± SE) h and the mean range in high and low temperatures for all storage periods tested was 1.6 ± 0.1 and 1.1 ± 0.0°C, respectively.

A relative humidity of 90% was attained after 1 d in storage in all replicates for each storage period and reached 100% shortly thereafter. Relative humidities of 90–95% are considered ideal storage conditions for table grapes (Mitchell 1985). Sulfur dioxide gas is liberated from the sodium bisulfite in the slow release pads under table grape storage conditions.

Sulfur dioxide concentrations in the foam containers ranged between 0.2 and 1.0 ppm during the 1- to 6-wk storage period (Table 2). For initial fumigation of grapes after harvest, the concentration multiplied by time product of 100 ppm · h is sufficient to control *Botrytis* spores (Smilanick and Henson 1992). For subsequent storage fumigations, 50 ppm · h should be used (Smilanick and Henson 1992). However, during ocean shipment for periods longer than 10 d in which tra-

Table 2. Slow release pad generated sulfur dioxide concentrations in grape cluster bags packed in foam containers with omnivorous leafroller in diet cups after 1 and 3 d, and 1–6 wk in low temperature storage (mean ± SE of 5 replicates)

Sulfur dioxide concn, ppm							
Days		Weeks					
1	3	1	2	3	4	5	6
0.5 ± 0.1	1.0 ± 0.4	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.7 ± 0.2

ditional sulfur dioxide fumigation cannot be applied the use of a sulfur dioxide generating pad and liner is recommended (Crisosto et al. 1996).

The sulfur dioxide concentrations generated from slow release pads in our tests (Table 2) were below the detectable odor concentrations of 30–40 ppm (Ryall and Harvey 1959) and would not cause phytotoxic effects (Crisosto et al. 1994) such as bleaching (Luvisi et al. 1992). The maximum safe workplace concentration of sulfur dioxide is 2 ppm (8-h time-weighted average) (Luvisi et al. 1992). The U.S. Environmental Protection Agency tolerance is 10 micrograms sulfur dioxide per gram of fresh grapes (Austin et al. 1997). Sulfite residue levels do not exceed 10 ppm on table grapes from sulfur dioxide slow release pad treatments (Crisosto et al. 1994).

The effect of low temperature storage combined with sulfur dioxide slow release pads on survival of 2nd instar omnivorous leafroller over a period of 6 wk is shown in Table 3. Complete control of the 2nd instars was obtained after 3 wk of exposure to the combination treatment. Low temperature storage alone caused only 99.0% mortality of the same instar and complete control was not attained even after 6 wk in storage at 0–1°C (unpublished data).

Acute exposures to high doses of sulfur dioxide were found to be toxic to omnivorous leafroller (Vail et al. 1992). In our study, omnivorous leafroller was controlled with very low doses of sulfur dioxide over a long period when combined with low temperature storage.

A combination treatment of low temperature storage and sulfur dioxide slow release pads offers an economical method to attain quarantine control of omnivorous leafroller in table grapes. Implementation of the procedure will not require new facilities or equipment because existing packinghouse cold storage operations and packaging techniques can be used.

Table 3. Percentage mortality of omnivorous leafroller 2nd instars based on adult survival after exposure to low temperature storage combined with sulfur dioxide slow release pads over a 6-wk storage period (mean ± SE of 3 replicates)

Storage, wk	*n*	% mortality
0	7,560	20.2 ± 4.6
1	2,520	62.8 ± 2.3
2	2,520	99.5 ± 0.4
3	2,520	100.0
4	2,520	100.0
5	2,520	100.0
6	2,520	100.0

The combination treatment also has potential for application during transit by ocean freight.

Based on our results, low temperature storage combined with sulfur dioxide slow release pads shows potential as a method to control omnivorous leafroller in exported table grapes. Other potential postharvest techniques such as controlled atmospheres also show promise as quarantine treatments (Mitcham 1997, Mitcham et al. 1997). These proposed control methods are alternatives to fumigants such as methyl bromide which may become obsolete for use as a quarantine treatment.

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