Pest Response in Packed Table Grapes to Low Temperature Storage Combined with Slow-Release Sulfur Dioxide Pads in Basic and Large-Scale Tests

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ABSTRACT The effect of low temperature storage combined with slow release sulfur dioxide pads was determined in basic laboratory and large-scale commercial tests on western flower thrips, *Frankliniella occidentalis* Pergande; grape mealybug, *Pseudococcus maritimus* (Ehrhorn); Pacific spider mite, *Tetranychus pacificus* McGregor; twospotted spider mite, *Tetranychus urticae* Koch; and omnivorous leafroller, *Platynota stultana* Walshingham. Temperatures within the foam containers among the packed clusters decreased from ambient to 2°C within approximately 1 d and ranged from 0.4 to 1.7°C in all tests. Sulfur dioxide concentrations in the foam containers ranged between 0.2 and 1.6 ppm during the 1- to 6-wk storage period in basic tests and 0.5–1.1 ppm during the 1- to 8-wk storage period in the large-scale test. Western flower thrips was completely controlled by a ≥1-wk exposure. Grape mealybug mortality was ≥93% after 2–5 wk exposures and 100% after a 6-wk exposure in basic tests. Pacific spider mite and twospotted spider mite mortality was 98.0 and 99.6%, respectively, after a 6-wk exposure. Mortality of grape mealybug and twospotted spider mite increased significantly at ≥3-wk exposures and Pacific spider mite mortality increased significantly at ≥4-wk exposures. Mortality of the spider mites in general was directly related to the duration of exposure. An 8-wk exposure to low temperature storage combined with slow release sulfur dioxide pads in the large-scale test resulted in 100% mortality of western flower thrips, twospotted spider mite, and omnivorous leafroller. The treatment resulted in <8% survival of grape mealybug and <1% survival of Pacific spider mite in the large-scale test. The combination treatment offers an economical method to attain quarantine control of certain insects and mites.

KEY WORDS *Platynota stultana, Pseudococcus maritimus Tetranychus urticae, Tetranychus pacificus, Frankliniella occidentalis, Vitis vinifera,* quarantine

A COMBINATION TREATMENT of low temperature storage and slow release sulfur dioxide pads was proposed for control of the omnivorous leafroller, *Platynota stultana* Walshingham, in packed table grapes, *Vitis vinifera* L., for export into areas where the pest is not found (Yokoyama et al. 1999). In addition to omnivorous leafroller, other pests of regulatory concern that have been reported to occur on grapes, include the western flower thrips, *Frankliniella occidentalis* Pergande, the grape mealybug, *Pseudococcus maritimus* (Ehrhorn), the Pacific spider mite, *Tetranychus pacificus* McGregor, and an occasional pest, the twospotted spider mite, *Tetranychus urticae* Koch. (Flaherty et al. 1992) The efficacy of the combination treatment on these additional pests has not been investigated.

Low temperature was studied as a quarantine treatment for the omnivorous leafroller (Yokoyama and Miller 2000), the western flower thrips (Carpenter et al. 1995), a mealybug on apples (Hoy and Whiting 1997), and a mite on grapes (Jadue et al. 1996). Low temperature as a single quarantine treatment could be economically implemented as a normal handling procedure or combined with other field and postharvest control practices to reduce the risk of accidental pest introductions (Yokoyama and Miller 2000). In cases of incomplete mortality, such as observed for omnivorous leafroller (Yokoyama and Miller 2000), low temperature was combined with the postharvest use of slow release sulfur dioxide pads (Yokoyama et al. 1999) to achieve a high level of pest control and quarantine security. Combination treatments may not always increase the efficacy of low temperature alone. Lester et al. (1997) found that storing diapausing twospotted spider mite for up to 8 wk at 0°C after immersion in 47°C water had no effect on mite mortality relative to mites assessed immediately after immersion.

The objective of this study is to confirm the efficacy of low temperature combined with slow release sulfur dioxide pads to control omnivorous leafroller in a large-scale commercial test, and to determine the efficacy of the combined treatment on western flower thrips, grape mealybug, Pacific spider mite, and twospotted spider mite in basic laboratory and large-scale tests.

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation by the USDA for its use.

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Materials and Methods

Source of Insects. 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). Omnivorous leafroller second instars were obtained by methods described by Yokoyama et al. (1999). A colony of western flower thrips was obtained from the Department of Entomology, University of California, Davis, CA, and reared on fresh green beans. All life stages of the grape mealybug were collected from a flame seedless grape vineyard in Dinuba, CA. A colony of Pacific spider mites on baby lima bean plants was obtained from Biotactics, Riverside, CA. A colony of twospotted spider mites on bean plants was purchased from Bio Ag Services, Fresno, CA.

Preparation of Insect and Mite Samples. Western flower thrips first and second instars, pupae, and adults were aspirated into 150-ml glass bottles (10 cm high by 5 cm diameter, ≈100–200 insects per bottle). The top of the bottle was covered with cotton organdy held in place with a rubber band. In basic tests, two bottles of different life stages were used in each of three replicates for each 1- through 6-wk exposure as follows: Replicate 1, one bottle of first instars and one bottle of pupae combined with adults; replicate 2, one bottle of second instars combined with pupae and one bottle of pupae combined with adults; and, replicate 3, one bottle of second instars combined with pupae and one bottle of adults. In the control, the number of bottles used for each life stage (≈100 insects per bottle) were as follows: first instar, two bottles; second instar combined with pupae, one bottle; pupae combined with adults, two bottles; and adults, two bottles.

In the large-scale test with western flower thrips, three clear plastic containers (7.2 cm high by 9.5 cm diameter with ≈750–3,400 insects per container) of different life stages were tested in two replicates as follows: Replicate 1, one container each of first instars, second instars, and adults; and, replicate 2, one container each of second instars, pupae, and pupae combined with adults. The top of the container was closed with a lid that had a 6-cm-diameter opening covered with nylon organdy. The basic test control was used for the large-scale test control.

Grape mealybug nymphs and adults (≈15) were placed in a cup (approximately 33 ml capacity, model P100, Solo, Urbana, IL) with a ruby seedless grape berry and held in plastic cup holder trays (30 cup capacity, BioServ, Frenchtown, NJ). The basic test included three replicates of one to two trays (30–60 cups with ≈450–900 insects per replicate) for each of 2- through 6-wk exposures. A control of three replicates of one tray (30 cups with ≈500 insects per replicate) was held in the laboratory at ≈23°C and never exposed to low temperature. Ruby seedless grape clusters infested with all life stages of grape mealybug were collected from a vineyard and used in each of two replicates of the large-scale test (six clusters per replicate, 90–800 nymphs and adults per cluster).

Pacific spider mite nymphs and adults (≈100) on a piece of leaf (≈1.5 cm wide by 1.5 cm long) cut from infested bean plants were placed in a cup and held in trays (30 cups per tray). The basic test included three replicates of one tray (30 cups with ≈2,000 mites per replicate) for each of 1- through 6-wk exposures. A basic test control of three replicates of one tray (30 cups with ≈2,000 mites per replicate) was held in the laboratory at ≈23°C and never exposed to low temperature. Seventeen trays (510 cups with ≈17,000 mites) were used in one replicate of the large-scale test. A large-scale test control of three replicates of one tray (30 cups with ≈1,000 mites per replicate) was held in the laboratory at ≈23°C.

Twospotted spider mite nymphs and adults (≈20) on a piece of leaf (≈1.5 cm wide by 1.5 cm long) cut from infested bean plants were placed in a cup and held in trays (30 cups per tray). The basic test included three to five replicates of one tray (30 cups with ≈650 mites per replicate) for each of 1- through 6-wk exposures. A control of two replicates of one tray (30 cups with ≈650 mites per replicate) was held in the laboratory at ≈23°C and never exposed to low temperature. Adults and nymphs (≈4,000) collected on white plastic cup lids (4.2 cm diameter, model LI-1, Plastics, St. Paul, MN) were used in a separate test.

Omnivorous leafroller second instars in diet (approximately 10 ml) in plastic cups were prepared according to Yokoyama et al. (1999) for the large-scale test. Thirty infested diet cups (≈10 insects per cup) were placed in a plastic cup holder tray. Thirty-four trays (1,020 cups with ≈10,200 second instars) were used in each of three replicates of the large-scale test. A control of three replicates of 11 trays (330 cups with ≈3,300 second instars) was held in an incubator (27°C and a photoperiod of 16:8 [L:D] h) and never exposed to low temperature.

Packing of Table Grapes with Insects and Mites. Ruby seedless grape clusters packaged in polyethylene cluster bags, ≈10 bags per box (8.6-kg box, Kings Canyon Fruit Sales, Reedley, CA) were transferred to a foam (expanded polystyrene) container (50 cm wide by 50 cm long by 17 cm high) lined with a perforated polyethylene box liner as described by Yokoyama et al. (1999).

In basic tests with western flower thrips and Pacific spider mite, one foam container packed with table grapes was used per replicate. In basic tests with grape mealybug and twospotted spider mite, two foam containers of packed table grapes were used per replicate. Three replicates were used for each 1- through 6-wk exposure. Two bottles of western flower thrips, one tray (30 cups) of grape mealybug or Pacific spider mite, or a half tray (15 cups) of twospotted spider mite were placed in each foam container at random among the grape clusters with separate foam containers (18 or 36 total) for each species.

The large-scale test included three replicates of 34 foam containers packed with table grapes. In replicates 1–3, 30 cups of omnivorous leafroller were placed among the grape clusters in every foam container. In replicates 1–2, either one grape cluster in-

Table 1. Time to attain 2°C from ambient air temperature among grape clusters with test insects and mites packed in foam containers after placement in low temperature storage (0–1°C), and storage temperatures during each exposure period thereafter in basic tests with western flower thrips, grape mealybug, and Pacific spider mite, and in the large-scale test with the same insects and omnivorous leafroller and two-spotted spider mite (mean ± SEM of 1.3 replicates per week for 6 wk)

Pest species	Time to 2°C, h	Storage temp. °C
Western flower thrips	22.1 ± 9.4	0.9 ± 0.2
Grape mealybug	21.5 ± 9.7	1.0 ± 0.2
Pacific spider mite	1.0 ± 0.0	0.4 ± 0.1
Omnivorous leafroller	25.2 ± 14.8	1.7 ± 0.04

fested with grape mealybug (three clusters per replicate) or one container of western flower thrips (three containers per replicate) were added to each foam container. In replicate 3, 15 cups of Pacific spider mite were added to each foam container. In a separate test, twospotted spider mite nymphs and adults on lids were placed in one foam container without grape clusters.

Monitoring Temperature and Relative Humidity. Temperature and relative humidity dataloggers (Stowaway, Intermountain Environmental, Logan, UT) were used in basic tests as described by Yokoyama et al. (1999). The sensor for each datalogger was placed among the berries in a cluster bag. The number of temperature dataloggers used in basic tests were as follows: Western flower thrips, one per replicate per exposure period; grape mealybug, one per each exposure period from 1 to 3 wk, one per replicate per exposure period from 4 to 6 wk; and Pacific spider mite, one per each of two replicates per exposure period. The large-scale test included three temperature and three relative humidity dataloggers placed at random in separate foam containers in each of three replicates. The time (hours) for the air temperature among the grape clusters to decrease to 2°C after placement in low temperature storage was reported as the mean ± SEM. The temperature in the packed foam containers after 2°C was attained was reported as the mean ± SEM of the replicates per week for 6 wk.

Exposure to Sulfur Dioxide and Low Temperature. In basic and large-scale tests, slow release sulfur dioxide pads containing anhydrous sodium bisulfite (7 g) (20 cm wide by 38 cm long, 12 packets per pad) (Grape Guard, Uvas, Santiago, Chile) were placed on an absorbent paper liner (26 cm wide by 37 cm long) on top of the grape clusters and test insects or mites 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 packed containers were treated with an initial fumigation (Luvisi et al. 1992) of sulfur dioxide (5,000 ppm for 30 min) in a commercial fumigation chamber, which is a normal prestorage procedure to reduce decay. The foam containers were aerated (approximately 1 h) and 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.

Determination of Sulfur Dioxide Concentrations. Sulfur dioxide concentrations for initial fumigations were determined with passive dosimeter tubes (No. 5D, Gastec, Ayase, Japan) placed in separate foam containers as follows: Basic tests with grape mealybug and twospotted spider mite, one tube; basic tests with western flower thrips or Pacific spider mite, three tubes; large-scale test replicate 3, three tubes. The tubes were removed upon completion of the initial fumigation and the sulfur dioxide concentration determined.

Sulfur dioxide concentrations in the foam containers with slow release sulfur dioxide pads were determined by methods described by Yokoyama et al. (1999). A detector tube (No. 5lb, Gastec, Ayase, Japan) was attached to polyethylene tubing with the opposite end placed in the center of the foam container. Gas samples were drawn through the detector tube with a pump (Matheson-Kitagawa model 8014–400A, Matheson Gas Products, Montgomeryville, PA).

In basic tests, concentrations were determined from each foam container (1–2 containers per each of three replicates) at the end of each exposure period. In the large-scale test, 21 foam containers were packed with table grapes but not insects or mites. These grapes were held in separate cold storage facilities so that the large-scale test with insects and mites were not disturbed. Three of these foam containers were removed from cold storage at the end of each weekly exposure period from 1 to 7 wk and the sulfur dioxide concentrations determined in each of the three replicate

Table 2. Sulfur dioxide concentrations (mean ± SEM of three replicates of one to six determinations per week for 6.8 wk) generated by slow-release pads among grape clusters packed in foam containers and stored at 0.4–1.7°C after 1–6 wk in basic tests, and after 1–8 wk in the large-scale test with omnivorous leafroller and the same insects used in basic tests

Pest species	Sulfur dioxide concn. ppm							
	Weeks							
	1	2	3	4	5	6	7	8
	Basic tests							
Western flower thrips	0.3 ± 0.1	0.3 ± 0.1	1.6 ± 1.0	0.3 ± 0.1	0.6 ± 0.3	1.1 ± 0.5		
Grape mealybug	1.5 ± 0.5	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.4 ± 0.1		
Pacific spider mite	0.7 ± 0.5	0.5 ± 0.2	1.4 ± 0.3	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.1		
Twospotted spider mite	1.0 ± 0.2	0.9 ± 0.3	0.7 ± 0.2	1.4 ± 0.5	0.3 ± 0.1	0.4 ± 0.0		
	Large-scale test							
Omnivorous leafroller	0.5 ± 0.1	0.8 ± 0.3	0.8 ± 0.3	1.1 ± 0.5	0.5 ± 0.1	0.6 ± 0.3	0.6 ± 0.4	0.5 ± 0.1

Table 3. Mean percentage mortality of insects and mites after exposure to low temperature storage (0.4–1.7°C) combined with slow release sulfur dioxide pads over a 6-wk storage period in basic tests

Pest species	n	Basic tests					
		Storage, wk					
		1	2	3	4	5	6
Western flower thrips[a]	1.698	100	100	100	100	100	100
Grape mealybug[b]	9.576		92.6 ± 2.1a	99.4 ± 0.6b	99.5 ± 0.2b	99.6 ± 0.2b	100b
Pacific spider mite[c]	28.782	54.0 ± 8.8a	57.1 ± 8.0a	78.5 ± 1.8a	86.3 ± 6.4b	94.4 ± 0.5b	98.0 ± 0.3b
Twospotted spider mite[d]	10.965		80.0 ± 8.7a	95.9 ± 1.7b	99.2 ± 0.3b	96.5 ± 3.2b	99.6 ± 1.0b

Means within a row followed by the same letter are not significantly different. $P > 0.05$. Tukey's test (GraphPad Software 1996).
[a] Three replicates of approximately 82–104 insects per storage period based on survival in controls.
[b] Three replicates of approximately 399–798 insects per storage period based on survival in controls.
[c] Three replicates of approximately 1.599 mites per storage period based on survival in controls.
[d] Three to five replicates of approximately 645 mites per storage period based on survival in controls.

containers. Upon completion of the three large-scale test replicates after 8 wk, six foam containers were selected at random from each of the three test replicates and the sulfur dioxide concentrations determined in each container. Concentrations (parts per million) were reported as the mean ± SEM of the replicates for each exposure period.

Evaluation of Mortality. Containers, cups, or clusters containing insects or mites were removed from the packed grapes at the end of each basic and large-scale test. Western flower thrips, grape mealybug, Pacific spider mite, and twospotted spider mite were evaluated for mortality. Insects or mites that showed movement were considered alive. Omnivorous leafroller second instars in diet cups were incubated (27°C and a photoperiod of 16:8 [L:D] h) with the controls for a minimum of 13 wk. Adults that emerged from the diet were considered survivors. Survival of immatures and adults in controls was used to estimate western flower thrips, grape mealybug, and twospotted spider mite populations in basic tests and Pacific spider mite in the basic and large-scale tests. Adult emergence in controls was used to estimate omnivorous leafroller test populations in the large-scale test. The total number of immature and adult western flower thrips and grape mealybug tested in the large-scale test was multiplied by the percentage survival in the basic test controls to determine the test population. The results for all tests were reported as the mean ± SEM percentage mortality of the replicates. Means of the treatment duration for each species were separated using Tukey's test (GraphPad Software 1996).

Table 4. Percentage mortality of insects and mites after exposure to low temperature storage (0.4–1.7°C) combined with slow release sulfur dioxide pads over an 8-wk storage period in the large-scale test

Pest species	n[a]	% mortality
Western flower thrips	4,738	100
Grape mealybug	3,566	90.4 ± 3.2
Pacific spider mite	15,878	99.7 ± 0.1
Twospotted spider mite	4,147	100
Omnivorous leafroller	23,256	100

[a] Based on survival in controls.

Results and Discussion

The air temperature was set at 0–1°C in the cold storage facilities used in basic and large-scale tests to determine the efficacy of cold storage combined with slow release sulfur dioxide pads to control insects and mites. Temperatures within the foam containers among the packed clusters decreased from ambient to 2°C within approximately 1 d in all tests (Table 1). Thereafter, in all tests, temperatures fluctuated between approximately 1 and 3°C. The time to attain treatment temperature and maintenance of such temperature to achieve efficacy is of quarantine importance.

A relative humidity of 90% was attained after 1 d in both basic and large-scale tests. Relative humidity was maintained at 100% thereafter. Sulfur dioxide gas is liberated by hydration of the sodium bisulfite in the slow-release pads under table grape storage conditions.

Passive dosimeter tubes showed >150 ppm·h sulfur dioxide concentrations indicating that initial fumigations were completed. Sulfur dioxide concentrations in the foam containers with slow release sulfur dioxide pads ranged between 0.2 and 1.6 ppm during the 1- to 6-wk storage period in basic tests and 0.5–1.1 ppm during the 1- to 8-wk storage period in the large-scale test and were similar to those previously reported by Yokoyama et al. (1999) (Table 2). The attributes of slow release sulfur dioxide pads to control decay under table grape storage and shipping conditions was discussed by Yokoyama et al. (1999).

The effect of low temperature storage combined with slow release sulfur dioxide pads on mortality of western flower thrips, grape mealybug, Pacific spider mite, and twospotted spider mite over a 6-wk exposure period in basic tests is shown in Table 3. Western flower thrips was completely controlled by a ≥1-wk exposure. However, Brodsgaard (1993) reported that 1% of western flower thrips adults and pupae are likely to survive 110.2–167.3 h at −5°C. Grape mealybug mortality was ≥93% after 2- to 5-wk exposures and 100% after a 6-wk exposure. Pacific spider mite and twospotted spider mite mortality was 98.0 and 99.6%, respectively, after a 6-wk exposure. Mortality of grape mealybug and twospotted spider mite increased sig-

nificantly at ≥3-wk exposures and Pacific spider mite mortality significantly increased at ≥4-wk exposures. Pacific spider mite may be more tolerant to the combination treatement than western flower thrips, grape mealybug, or twospotted spider mite. Mortality of grape mealybug and the spider mites was directly related to the duration of exposure.

An 8-wk exposure to low temperature storage combined with slow release sulfur dioxide pads in the large-scale test resulted in 100% mortality of western flower thrips, twospotted spider mite, and omnivorous leafroller (Table 4). The treatment resulted in <8% survival of grape mealybug and <1% survival of Pacific spider mite.

Complete control of western flower thrips in the large-scale test (Table 4) was consistent with basic test results, which showed the insect was susceptible to exposures as short as 1 wk (Table 3).

Grape mealybug did not survive a 6-wk exposure in basic tests (Table 3) but survived an 8-wk exposure in the large-scale test. The difference may be attributed to testing methodologies. Grape mealybug in basic tests were exposed in plastic cups and in the large-scale test were exposed in a natural state in clusters. The webbing and protective niches of the cluster may have contributed to survival in the large-scale test.

Pacific spider mite was not completely controlled by an 8-wk exposure. The low level of survival observed in the large-scale test suggests that extending the treatment time may provide complete control. Twospotted spider mite was completely controlled by an 8-wk exposure in the large-scale test. However, these mites were exposed on plastic lids without protected niches provided by leaves, which may have enhanced mortality. The large-scale test confirms the efficacy of the treatment to control omnivorous leafroller. Confirmation of 100% mortality of ≈30,000 insects shows that the treatment provides quarantine security.

A combination treatment of low temperature storage and slow release sulfur dioxide pads offers an economical method to attain quarantine control of certain insects and mites. Implementation of the procedure will not require new facilities or equipment because existing packinghouse cold storage operations and packaging techniques can be used. The combination treatment has great potential for application during transit by ocean freight.

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