Ultralow Oxygen Treatment for Control of *Latrodectus hesperus* (Araneae: Theridiidae) on Harvested Table Grapes

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ABSTRACT The spider *Latrodectus hesperus* Chamberlin & Ivie (Araneae: Theridiidae) was subjected to low and ultralow oxygen (ULO) treatments at different temperatures. Complete control of the spiders was achieved in 24-h ULO treatments with 0.5% O_2 or lower at 1°C and in a 24-h low oxygen (2%) treatment at 15°C. Oxygen level and temperature greatly affected spider mortality. At 1°C, as oxygen level was decreased from 2 to 0.5%, spider mortality increased from 0 to 100%. At 2% O_2, as temperature was increased from 1 to 15°C, spider mortality increased from 0 to 100%. Grape clusters from two table grape (*Vitis* spp.) cultivars, 'Thompson Seedless' and 'Flame Seedless', were subjected to the 24-h ULO treatment with 0.5% O_2 at 1°C. The ULO treatment had no negative effects on grape quality. Because of the relatively short treatment time, effectiveness at low storage temperature and the easily attained oxygen level, we conclude that the ULO treatment have good potential to be implemented commercially for control of black widow spiders on harvested table grapes.

KEY WORDS black widow spider, table grape, postharvest pest control, controlled atmosphere, ultralow oxygen

The spider *Latrodectus hesperus* Chamberlin & Ivie (Araneae: Theridiidae) has been found in table grape (*Vitis* spp.) clusters exported from California to overseas markets; therefore, it poses a great concern for the California table grape industry. To control the spiders, exported table grapes bound for Australia and other overseas markets are fumigated with 6% CO_2 plus 1% SO_2 for 30 min at 15.6–20.6°C (Mitcham et al. 2005). This is in addition to methyl bromide fumigation to control other arthropod pests that might be found on table grapes. However, these two required fumigation treatments were found to have a negative impact on the quality of 'Thompson Seedless' table grapes, especially when warmer holding temperatures were used in combination with the SO_2 and methyl bromide fumigation treatments (Mitcham et al. 2005). Methyl bromide depletes atmospheric ozone; thus, its production and use are being phased out globally as mandated by the Montréal Protocol. Although its use for pest quarantine treatment is exempt from phasing out at present, there is no guarantee that this status will not change in the future. Its global production phase out also will likely make methyl bromide fumigation very expensive in the future. Therefore, alternative treatments are needed to control black widow spiders and other arthropod pests on exported table grapes.

There are five species of black or brown widow spiders recognized in North America (Garb et al. 2004). *L. hesperus* occurs in California, and it is commonly found in grape vineyards (Daane et al. 2004). The spider is wary of any movement and quickly withdraws into protected sections of the vine or trellis structure when disturbed. At harvest, a small percentage of the black widow spider population will reside or move into the grape clusters and be inadvertently packed with the table grapes destined for market and thereby create a safety hazard to consumers. Therefore, postharvest treatment of table grapes to control black widows is important.

Controlled atmosphere (CA) has been studied as an alternative to methyl bromide fumigation for postharvest pest control on exported fresh commodities (Mitcham et al. 2003). CA treatments typically use reduced oxygen or/and elevated carbon dioxide to control pests. But the progress in the development of CA treatments has been hampered by adverse effects on the quality of treated commodities (Mitcham et al. 2003). There is also no information on susceptibility of black widow spiders to CA treatments. Atmosphere with ≤1% O_2 is referred as ultralow oxygen (ULO). CA treatment with ultralow oxygen (ULO treatment) has been studied in recent years for postharvest insect control on lettuce and broccoli (Liu 2005, 2007, 2008). In this study, we determined effects of ULO treatments with different oxygen levels and temperatures

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on survival of black widow spiders and quality of table grapes.

Materials and Methods

Black Widow Spiders. A laboratory culture of *L. hesperus* was established from egg sacs collected in Fresno and Salinas, CA, as well as from a laboratory colony maintained at the University of California's Kearney Research and Educational Center near Parlier, CA. Upon hatching from egg sacs, spiderlings were transferred into plastic vials (7 cm in length by 3 cm in diameter) using a paint brush (five spiderlings per vial). The spiderlings were allowed to cannibalize, and this resulted in one survivor in each vial. Thereafter, the spiders were fed twice a week with Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), larvae, which were reared on a wheat bran insect diet (Tebbets et al. 1978). The vials with medium- to large-size black widow spiders (4–6 mo after hatching) were used for treatments.

Effects of Low and Ultralow Oxygen Treatments on Survival of Black Widow Spiders. Black widow spiders were subjected to 24-h low oxygen and ULO treatments in plastic jars at different temperatures. Oxygen levels of 0.2, 0.5, 1.0, and 2.0% were tested at 1°C and the 2.0% O_2 also was tested at 10 and 15°C. Small holes were punched into the lids of the plastic vials housing the spiders to allow adequate air exchange during the treatments. The plastic vials were sealed in plastic treatment jars (15 cm in height by 9 cm in diameter) with lids. Each lid had two ports to allow airflow in and out of the jar. The ULO treatment with 0.5% O_2 was replicated four times. The 1 and 2% O_2 treatments were replicated two times. The treatment with 0.2% O_2 was not replicated. In total, 91 spiders were tested in the six treatments. The spiders were held in jars at 1, 10, and 15°C in fresh air as controls.

The low oxygen and ULO treatments were accomplished by passing a mixture of nitrogen with 0.2% oxygen from a nitrogen generator (Balston 75-7820, Parker Hannifin Co., Tewksbury, MA) with compressed air through the treatment jars; flow meters were used to produce a controlled flow rate of 0.5–0.8 liter/min. The outlet of each jar led into a jar with water, which allowed air in the treatment jars to exit while still maintaining positive pressure in the treatment jars. The oxygen levels in the treatment jars were monitored with an oxygen analyzer (Series 800, IL Instruments, Inc., Johnsburg, IL). The tests were conducted in temperature cabinets with external digital temperature controls that maintained temperature variation within ±0.5°C. After treatment, spider mortality was scored after being held overnight at 22°C in an environmental chamber. Dead black widow spiders typically had legs fully extended downward from the body and hung loosely at a thread or fell to the bottom of the vial. Live spiders remained hanging from the web in their typical inverted position with their legs spread outward from the body, and moved when disturbed. Likelihood ratio chi square test was used to analyze the frequencies of live and dead spiders in response to low oxygen and ULO treatments by using JMP Statistical Discovery software (SAS Institute 2002).

Effects of Ultralow Oxygen Treatment on Table Grape Quality. The 24-h ULO treatment with 0.5% O_2 at 1°C was tested on table grapes together with black widow spiders to determine the effects on grape quality and verify its efficacy in controlling black widow spiders. Fruit clusters of the common table grapes Thompson Seedless and 'Flame Seedless' were obtained from local supermarkets. Grapes were screened for any defects before being subjected to the ULO treatment. For each test, grape clusters were paired by similar appearance, placed in paper bags and then randomly assigned to the control and the ULO treatment.

Black widow spiders in the rearing vials were placed inside each bag, next to the grape clusters, and the bag was then sealed in a box chamber (21 by 37 by 57 cm). The chamber had a circulation fan to circulate air constantly, a watertight seal to maintain treatment integrity, and two ports for air exchange. The ULO treatment was established by passing a mix of generated nitrogen with ≈0.2% O_2 and compressed air, as described above. The oxygen analyzer was connected to the second port to constantly monitor oxygen level in the chamber. At the end of each test, spiders in vials were held in an environmental chamber overnight before being scored for mortality, as described above. Grapes from the ULO treatment and the control were stored at 2°C for 2 wk and then evaluated for visual quality. The visual quality of berries was based on fruit decay, color, and firmness and scored on a 1–4 scale, where 1 indicated unmarketable; 2, poor to medium; 3, good or marketable; and 4, excellent. Percentages of premium-quality berries from both the treatment and the control were calculated. Observations also were made on rachis color of grape clusters from the treatment and the control. The treatment was replicated two times, and 20 black widow spiders in total were treated. The differences in the visual quality of grapes and the percentages of premium quality berries between the treatment and the control were analyzed using Student *t*-test (SAS Institute 2002).

Results and Discussion

Complete control of black widow spiders was achieved in the 24-h ULO treatments with ≤0.5% O_2 at 1°C and the 24-h low oxygen treatment with 2% O_2 at 15°C (Table 1). Mortality data at 0.5% O_2 were pooled from the 34 spiders tested in plastic jars and the 20 spiders tested with table grapes in data analysis. There was a negative relationship between oxygen level and spider mortality: as the oxygen level was decreased from 2 to 0.5% at 1°C, the spider mortality increased from 0 to 100%. For 0.5% O_2 and 1.0% O_2 treatments at 1°C, the mortality difference was highly significant based on likelihood ratio chi-square test ($\chi^2 = 24.472, P < 0.0001$). All spiders survived 24-h low oxygen treatment with 2% oxygen at 1°C. There was also a positive relationship between temperature and

Table 1. Effects of 24-h ultralow oxygen treatments on survival of *L. hesperus*

Treatment		Spiders tested	Spider mortality (%)
O2 level (%)	Temp (°C)		
0.2	1	5	100
0.5	1	54	100
1.0	1	14	57.1
2.0	1	14	0
2.0	10	12	16.7
2.0	15	12	100

There was a significant difference in the frequency of dead and live spiders for the 0.5% O_2 and 1.0% O_2 treatments ($\chi^2 = 24.472$, $P < 0.0001$), and there were also significant differences in the frequency of dead and live spiders among the three 2.0% O_2 treatments at three different temperatures ($\chi^2 = 39.203$, $P < 0.0001$) (SAS Institute 2002).

spider mortality at the 2% O_2 level: as temperature was increased from 1 to 15°C, spider mortality increased from 0 to 100% (Table 1). The mortality changes for the 2.0% O_2 treatments at three different temperatures were highly significant based on likelihood ratio chi-square test ($\chi^2 = 39.203$, $P < 0.0001$).

The 0.5% ULO treatment (24 h at 1°C) had no negative effect on grape quality. There was no significant difference in quality score between the treatment and the control ($t = 0.00$; df = 1, 6; $P = 1.00$) (Table 2). Quality ratings of grapes from both the ULO treatment and the control were between 3 (good) and 4 (excellent), with slightly higher scores for the red Flame Seedless than the green Thompson Seedless grapes. Similarly, there was no significant treatment effect on the percentage of premium-quality berries ($t = -1.352$; df = 1, 5.97; $P = 0.23$) (Table 2). The Thompson Seedless had ≈86 and 79% premium quality berries for the ULO treatment and the control, respectively, whereas the Flame Seedless had ≈94 and 95% premium-quality berries for the ULO treatment and the control, respectively (Table 2). There was also no difference in rachis color between the treatment and the control. These results were expected as we had observed that table grapes tolerated ULO treatments with much lower O_2 levels at 1°C for several days (Y.-B.L., unpublished data). The oxygen level of 0.5% in the ULO treatment for black widow spider control was not far from the oxygen level of 2–5% recommended for long-term CA storage of table grapes; therefore, the ULO treatment could be easily achieved in commercial CA storage facilities. This suggests that the ULO treatment can be a practical and economical solution to the black widow spider problem on exported table grapes.

Table 2. Effects of ULO treatment of 0.5% O_2 at 1°C for 24 h on quality of table grapes, after 2-wk storage

Grape cultivar	Treatment	Berries rated	Quality score	Marketable fruit (%)
Thompson Seedless	ULO	310	3.5	86.4 ± 4.6
	Control	372	3.5	78.6 ± 9.6
Flame Seedless	ULO	769	4.0	93.6 ± 3.1
	Control	566	4.0	94.9 ± 1.9

There was no significant difference in the quality score or the percentage of premium quality berries between the ULO treatment and the control ($P > 0.05$, Student *t*-test; SAS Institute 2002).

Previous studies have shown that, for some arthropods, ULO treatment has the promise to be used effectively for postharvest insect control on perishable commodities (Mitcham et al. 2003; Liu 2005, 2007, 2008). In comparison with the previously tested insect species, which may have required ULO treatment with as low as 0.003% O_2 for several days (Liu 2005, 2007, 2008), *L. hesperus* was far more susceptible to ULO treatments than any insect species. We suspect that the high susceptibility of *L. hesperus* to the low oxygen and ULO treatments lies in the morphological differences in their respiratory systems.

Black widow spiders have an open blood circulation system with all organs surrounded by blood. They possess book lungs in the abdomen for air exchange and have tracheas with openings above the spinners connecting to the book lungs (Gillespie and Spagna 2003). Oxygen is transported by a complex protein called hemocyanin through circulating blood to reach various organs and cells. Blood circulation is also aided by spider movement (Anderson and Prestwich 1985). The hemocyanin is only ≈5% efficient compared with hemoglobin in mammals in transporting oxygen (Gillespie and Spagna 2003). In comparison, insects, in general, have well developed tracheal systems for gas exchange. Oxygen enters insect bodies via spiracles and diffuses through branching tracheae and tracheoles to reach body fluid within organs and muscles (Harrison 2003). Therefore, it is possible that black widow spiders have much less efficient mechanism in transporting oxygen than insects; thereby, they are more prone to the stress of oxygen deficiency.

In summary, the ULO treatments with 0.5% O_2 or lower at 1°C for 24 h and a low oxygen treatment of 2% O_2 at 15°C for 24 h achieved complete of *L. hesperus.* The 0.5% O_2 ULO treatment was also tested on table grapes and did not have any negative impact on grape quality. The treatment was relatively short (24 h), and it was effective at low temperature (1°C) typically used for storage of table grapes. Therefore, the treatment is promising to be used to control black widow spiders on exported table grapes.

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