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Postharvest fumigation of California table grapes with ozone to control Western black widow spider (Araneae: Theridiidae)

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ABSTRACT

Ozone fumigations were evaluated for postharvest control of Western black widow spider (BWS), *Latrodectus hesperus* (Chamberlin and Ivie), in fresh table grapes destined for export from California USA. Mature adult female black widow spiders were contained in separate gas-permeable cages within a flow-through vacuum chamber and exposed for 1 h at 3 ± 1 °C ($\bar{x}$ ± s) and 67.5 kPa to gaseous ozone at eight steady-state concentrations (i.e, $[O_3]_{ss}$) over the range 0.71 ± 0.04–16.91 ± 0.28 g m^{-3} ($\bar{x}$ ± s) (i.e., 500 ± 25–12,000 ± 200 ppmv (μL L^{-1})) with or without supplementation of atmospheric carbon dioxide levels, respectively 129.3 or ca. 0.8 g m^{-3} $[CO_2]_{ss}$ (i.e., 100,000 or ca. 600 ppmv). Regression models of the concentration-mortality response predict 98% mortality of adult female BWS populations following ozone fumigation for 1 h at 3 ± 1 °C when headspace $[O_3]_{ss}$ is maintained at ~14 g m^{-3} (i.e., 10,000 ppmv) with ambient atmospheric CO_2 levels ($[CO_2]_{ss}$ = 0.8 g m^{-3}). Providing evidence to support the use of these treatment parameters for control of BWS in packed table grapes, 0 survivors were observed from 268 total specimens treated in a series of confirmatory fumigations conducted for 1 h at 3 ± 1 °C and 67.5 kPa with headspace $[O_3]_{ss}$ and $[CO_2]_{ss}$ maintained ≥14 and ca. 0.8 g m^{-3}, respectively.

1. Introduction

The Western black widow spider (BWS), *Latrodectus hesperus* (Chamberlin and Ivie), is a pest of concern to California USA fresh table grape growers and shippers (Kaston, 1970; Webster, 1979). Considered a beneficial species in the vineyard, BWS preys upon a myriad of insect pests that decrease productivity due to leaf defoliation and damage to berries. However, several countries that import table grapes from California cite BWS on their Pest Risk Analysis and either informally request, or formally require, phytosanitary measures to control adult females, which are much larger and relatively more aggressive toward prey than males and juveniles. To mitigate the potential for introduction of BWS into the marketing channel, various postharvest treatments have been proposed, such as fumigation with methyl bromide, phosphine, or a sulfur dioxide–carbon dioxide mixture (Mitcham et al., 2005; Department of Agriculture, Fisheries and Forestry (DAFF), 2013).

The use of gaseous ozone as a pesticidal fumigant at ppbv (nL L^{-1}) to ppmv (μL L^{-1}) concentrations is well established (Leesch and Tebbets, 2008; Palou et al., 2007; Metzger et al., 2007; Erdman, 1980; Leesch, 2003; Kells et al., 2001) and has been commercially explored. Collectively, these studies indicate that the phytotoxic impact of ozone fumigation needs to be evaluated for each commodity. Grape berry tolerance to ozone has been documented for several varieties while variable phytotoxicological consequences to grape cluster rachis has generally been observed (Cayuela et al., 2009; Luchsinger et al., 1999; Mlikota Gabler et al., 2010; Shimizu et al., 1982; Walse and Karaca, 2011).

We report the mortality of adult female BWS following exploratory fumigations with steady-state concentrations of gaseous ozone (i.e, $[O_3]_{ss}$), over the range 0.71 ± 0.04–16.91 ± 0.28 g m^{-3} ($\bar{x}$ ± s) (i.e., 500 ± 25–12,000 ± 200 ppmv (μL L^{-1})), maintained for 1 h at 3 ± 1 °C ($\bar{x}$ ± s) and 67.5 kPa. The effect of increasing the steady-state carbon dioxide level in chamber headspace (i.e., $[CO_2]_{ss}$) from ca. 0.8 g m^{-3} (600 ppmv; 0.04% CO_2 as in ambient atmospheric air) to 129.3 g m^{-3} (100,000 ppmv; 10% CO_2 in air) was evaluated, as supra-atmospheric CO_2 levels have been shown to enhance the efficacy of fumigants, including ozone, to certain insect species (Bond and Buckland, 1978; Cotton and Young, 1929; Kashi and Bond, 1975; Leesch, 2003; Wigglesworth, 1965). Respective to each of the two $[CO_2]_{ss}$ levels, $[O_3]_{ss}$ concentration-mortality responses were modelled in order to predict the fumigation parameters required for a particular mortality, and then the predictions were tested in a series of confirmatory trials.

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2. Materials and methods

2.1. Insects

Adult female Western BWS were collected on summer nights from evergreen bushes lining streets in the Northwestern residential area of Fresno, California. Each specimen was placed in a 30-mL clear plastic cup with a white cap (Tyco® #PI-1 cup, LI-1 cap, Minneapolis, Minnesota, USA), which were then grouped in a cloth sac. Typically, 100 to 150 specimens were captured per collection night. The following morning, all specimens were transported to the laboratory at the USDA-ARS-SJVASC (Parlier, California USA). Each specimen was transferred into a 7-dram plastic "snap cap" cage (Plastainer® #SP-37, Perrysburg, Ohio, USA) modified with 1 mm diameter holes (i.e., gas-portals) on the bottom, snap cap, and cylinder. Specimens were incubated in a 15.2-m^3 rearing unit (24–27 °C, 80% RH, 16:8 [L:D] h) and fed one or two, 4th to 5th instar *Amyelois transitella* or *Plodia interpunctella* larvae prior to conducting fumigations, which typically occurred within 1 to 3 days of collection.

2.2. Chamber fumigation system

Ozone fumigations were performed in a cylindrical stainless-steel chamber (25 cm diameter × 56 cm deep = 27.5 L volume) manufactured by Tahoe Foods Technology (Reno, Nevada, USA) that was equipped with a Magnehelic gauge (Cat. No. 2020C, Dwyer Instruments, Michigan City, Indiana, USA) as well as inlet and exhaust portals. Chamber temperature was maintained at 3 ± 1 °C. Flow from respective 300-lb source cylinders of breathing air and carbon dioxide (Airgas, Fresno, California, USA) were each metered with a rotameter and directed into a corona discharge unit (Clear Water® Model CD12 Ozone Generator, San Luis Obispo, California, USA) connected to an inlet port of the treatment chamber. $[O_3]_{ss}$ was tuned to the desired level by adjusting the electrical current (microamperage) of the corona discharge and/or metering the air input over the range 100 mL min^{-1} ($[O_3]_{ss}$ = 0.71 ± 0.04)–1.5 L min^{-1} ($[O_3]_{ss}$ = 16.91 ± 0.28 g m^{-3}). $[CO_2]_{ss}$ was tuned to either 129.3 g m^{-3} (100,000 ppmv; 10% CO_2 in air) or ca. 0.8 g m^{-3} (600 ppmv; 0.04% CO_2 as in ambient atmospheric air) by metering the CO_2 input. For all treatments, effluent flow through the exhaust port was metered with a vacuum of 33.8 kPa and directed through a manganese dioxide "scrubber". $[O_3]_{ss}$ was continuously measured by looping chamber headspace through an ozone gas analyzer (OzoMeter™ Model HC-HA-100-GTP-12, Hankin Ozone Systems Inc., Mountainview, California, USA). $[CO_2]_{ss}$ and oxygen concentration in chamber headspace were measured with a gas sampling pump connected in series with an atmospheric gas analyzer (Model 902D O2/CO2 Headspace Analyzer, Quantek Instruments, Inc., Grafton, Massachusetts, USA) and recorded at standard temporal intervals over the duration of treatment.

2.3. Exploratory fumigations

The mortality of adult female BWS was evaluated following fumigations in which $[O_3]_{ss}$ was varied over the range 0.71 ± 0.04–16.91 ± 0.28 g m^{-3} ($\bar{x}$ ± s).

Test specimens as well as untreated control specimens were allowed to equilibrate to treatment temperature (i.e., tempered) of 3 ± 1 °C for 18 h prior to fumigation. For each trial, ten cages, each containing a test specimen, were introduced into the chamber described above and the chamber door was closed. $[CO_2]_{ss}$ and $[O_3]_{ss}$ were tuned to the desired levels, which was typically accomplished in < 5 min; this marked the beginning of the 1-h treatment duration.

After fumigation, valves were opened to atmosphere and the chamber was aerated until 0 ppmv $[O_3]_{ss}$ was measured. The chamber was opened, treated as well as untreated control specimens were collected, and all specimens were transferred into respective 0.03-m^3 nylon-mesh rearing cubicles maintained in an incubator at 27.0 ± 1.0 °C and 60 ± 2% RH ($\bar{x}$ ± s).

Mortality evaluations were conducted ~24 h post fumigation. Survivability was diagnosed by locomotion or by prodding-induced motion. Specimens were categorized as moribund if the survivability was inconclusive. Moribund specimens were placed inside a labelled plastic snap-cap cage with a food source, as described above, and further incubated until an additional evaluation the following day 48 h after treatment.

The lethal response was profiled over eight different levels of $[O_3]_{ss}$, each replicated 3–5 times, with 10 spiders per trial (replicate). Probit analyses (Finney, 1971) of the response data were modelled using Polo Plus (LeOra Software LLC, 2002–2007) followed with a significance test comparing the two levels of $[CO_2]_{ss}$. Mortality of control specimens was included as a natural response in the efficacy modelling. The total number of specimens treated across exploratory-trials was estimated by summing the numbers from each respective trial.

2.4. Confirmatory fumigations

Three fumigation trials were conducted in the ozone chamber described above to verify the results of the exploratory fumigations. Adult female BWS test specimens as well as untreated control specimens were allowed to equilibrate to treatment temperature at 3 ± 1 °C for 12 h prior to fumigation. Caged test specimens, totaling 80 to 100 per fumigation replicate, were transferred into the chamber and the door secured shut. $[CO_2]_{ss}$ and $[O_3]_{ss}$ were tuned to the desired levels, which was typically accomplished in < 5 min; this marked the beginning of the 1-h treatment duration. Other fumigation procedures were as above. Mortality evaluations were conducted at 24 and 48 h post-fumigation as described above.

3. Results and discussion

Concentration-mortality regressions from 1-h ozone fumigations at 3 ± 1 °C and 67.5 kPa with $[CO_2]_{ss}$ of either 129.3 or ca. 0.8 g m^{-3} (i.e., 100,000 or ca. 600 ppmv, respectively) are shown in Fig. 1. The number of specimens treated, the number of control specimens included as a natural response in the model (all survived), the projected concentrations to cause 90, 95, and 99% mortality in the treated population (respectively LC_{90}, LC_{95}, LC_{99}), and the corresponding estimates of the bounds (upper (UL) and lower (LL) limits) at the 95% confidence level (CL) are listed (Fig. 1). Likelihood ratio-based hypothesis testing of equality and parallelism were not rejected (equality: $P > 0.05$, $\chi^2 = 4.8$, $df = 2$; parallelism: $P > 0.05$, $\chi^2 = 0.06$, $df = 1$), indicating that the predicted slopes and the intercepts were the same when comparing responses associated with $[CO_2]_{ss}$ of 129.3 versus ca. 0.8 g m^{-3}. Lethal concentration ratios (LCRs) were calculated with (±) 95% confidence intervals (CI) across the $[O_3]_{ss}$ concentrations predicted to cause 10 to 99% mortality in the treated populations. The LCRs, normalized to the response toward $[CO_2]_{ss}$ of ca. 0.8 g m^{-3}, paralleled a ratio of ca. 1 at durations predicted to cause > 80% mortality, indicating that $[CO_2]_{ss}$ levels investigated in this study did not significantly affect mortality (Fig. 2). These results provide evidence to support the conclusion that 98% mortality in adult female BWS will result following ozone fumigation for 1 h at 3 ± 1 °C, 67.5 kPa, and ca. 0.8 g m^{-3} (600 ppmv, 0.04% CO_2 as in ambient atmospheric air) when headspace $[O_3]_{ss}$ is maintained at ~14 g m^{-3} (i.e., 10,000 ppmv) (LC_{98}:14.48; LL 95% CL: 10.49; UL 95% CL: 22.99 g m^{-3}).

Three confirmatory fumigations were conducted to verify the predictions of the concentration-response models. While no mortality was observed in any of the 15 untreated control specimens respective to each trial, the confirmatory trials yielded 0 survivors from a total of 268 treated specimens (100, 86, and 82 treated respectively in trials 1, 2, and 3), results that provide evidence to support the use of ozone