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Tolerance of codling moth, and apple quality associated with low pressure/low temperature treatments

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ABSTRACT

A combination of low pressure (LP) and low temperature (LT) may serve as a phytosanitary disinfestation treatment for fresh fruit. In this study, different life stages of codling moth (eggs, 2nd to 3rd instar larvae, 5th instar larvae and pupae) were treated in hypobaric chambers maintained at 10 °C and 1.33 kPa with nearly saturated humidity (>98%). Weight loss, color, firmness, titratable acidity (TA), and soluble solids content (SSC) were selected as quality parameters to evaluate the quality changes of 'Red Delicious' apples before and after the LPLT treatment. Results showed that the 5th instar larvae were the most tolerant life stage for codling moth under LPLT treatment conditions. Insect mortality increased with increasing LPLT treatment time to >98% after 12 days of exposure to 10 °C temperature and 1.33 kPa pressure. Although stored in less than optimum conditions for apples, the measured quality variables of 'Red Delicious' were maintained relatively well after 15 days of LPLT treatment. The results suggest that LPLT technology has potential as an alternative, non-chemical disinfestation treatment for apples.

1. Introduction

Codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), a key pest of fresh fruit such as apples and pears (Witzgall et al., 2008), is an important phytosanitary and quarantine pest for many countries (Wang et al., 2004). Quarantine treatments using the chemical fumigant methyl bromide (MeBr) have been commonly required for importing countries to prevent the spread of this pest. Due to the ozone depletion potential of MeBr, its use is being phased out under the Montreal Protocol (UNEP, 2009). Most applications of MeBr have been banned in developed countries since the end of 2005 and will be banned worldwide by the end of 2015 (UNEP, 2009). Although quarantine and pre-shipment (QPS) treatments such as those targeting codling moth in fresh fruit, are currently exempt from restrictions under the Montreal Protocol, there is increasing pressure to extend the ban to these applications. Consequently, it is necessary to explore alternative non-chemical disinfestation treatment methods.

Many disinfestation treatment methods have been considered as alternatives to MeBr, such as hot air or hot water treatments, radio frequency (RF) treatment, ionizing irradiation treatment, cold storage, controlled atmosphere storage and other fumigants (Heather and Hallman, 2008). Low-pressure technology has also been proposed as an alternative disinfestation treatment for agricultural products since it is organic, residue free and environmentally sustainable (Bare, 1948; Calderon et al., 1966; Calderon and Navarro, 1968; Mbata and Phillips, 2001; Navarro et al., 2001; Davenport et al., 2006; Johnson and Zettler, 2009). Most of the early studies on low-pressure technology for insect disinfestation focused on its use in durable commodities at relatively high temperatures (ambient or above) (Calderon et al., 1966; Mbata and Phillips, 2001; Navarro et al., 2001; Johnson and Zettler, 2009). In contrast, much of the research on low-pressure treatments for fresh fruit has been to maintain product quality while extending storage life. Those studies were conducted at low temperatures and high humidities (Burg, 2004). At low temperatures, longer exposures are needed to obtain adequate insect control with low pressures (Mbata and Phillips, 2001; Mbata et al., 2004, 2005). However, because low-pressure/low-temperature (LPLT) technology provides extended storage times for fresh fruit, it has the potential

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to be used as an alternative disinfestation treatment (Davenport et al., 2006).

The insecticidal mechanism of low-pressure treatments is thought to be primarily due to the effects of reduced oxygen levels (Navarro and Calderon, 1979). Soderstrom et al. (1990) reported the relative responses of different codling moth life stages to low oxygen conditions (0.5% O_2 and 10% CO_2 at 25 °C with 60% or 95% relative humidity), noting that diapausing larvae were the most tolerant. Johnson and Zettler (2009) investigated the response of three lepidopteron postharvest pests in tree nuts (codling moth, navel orangeworm and Indianmeal moth) under a low-pressure (6.67 kPa) environment at 25 and 30 °C temperatures. Information on the relative tolerance of different codling moth life stages under LPLT treatment conditions (low pressures, low temperatures and high humidity) is lacking. It is important to determine the most tolerant codling moth life stage under LPLT treatment conditions, and this stage must be used in developing and validating LPLT disinfestation treatment protocols.

In addition to determining the efficacy of the treatment in controlling the target pest, it is also necessary to determine the treatment effect on the product quality. When evaluating the stability and performance of the LPLT system under different pressures, Jiao et al. (2012) showed that the LPLT system had the ability to control the pressure within 1% of the set point and maintained relative humidity at a nearly saturated level (>98%). Oxygen concentrations could be controlled at low levels (<0.6%) when the pressure was less than 3.33 kPa. The leakage rates of the hypobaric chamber and of the entire LPLT system were 0.01 and 0.48 kPa/h, respectively, and were considered acceptable. The demonstrated performance of the LPLT system (Jiao et al., 2012) provides a solid basis for the current insect tolerance and fruit quality study.

The main objective of the present study was to investigate the feasibility of using the LPLT technique to control insects and maintain the quality of fresh fruit. The lab-scale LPLT system tested previously was used to study the tolerance of codling moth at different life stages under the LPLT treatment environment, and apple quality was evaluated before and after the LPLT treatments.

2. Materials and methods

2.1. LPLT systems

A lab-scale LPLT system (Atlas Technologies, Port Townsend, WA, USA) with two identical hypobaric aluminum chambers (0.61 L × 0.43 W × 0.58 H m^3) was used in the current study. The system was equipped with a rotameter (Model FL-3841G, OMEGA Engineering Inc., Stamford, CT, USA) to adjust the air exchange rate, which was used to prevent buildup of metabolic gases given off by the fruit. A humidifier was used to make sure the inflowing rarefied air was humidified before entering the hypobaric chamber. Sensors inside the hypobaric chambers were used to record the temperature, humidity and pressure during treatment. The chamber system, housed in cold-storage room, was covered by flexible insulation sheets with a thickness of 0.013 m to reduce the temperature variations of the hypobaric chamber walls. Detailed information about the LPLT systems and instrumentation used in this study can be found in Jiao et al. (2012).

2.2. Insect mortality

Initial stock codling moths were obtained in 1984 from an apple orchard in Madera County, CA and reared at the San Joaquin Valley Agricultural Sciences Center (SJVASC). Test insects were reared at 27 °C, 60% RH and a photoperiod of 16:8 (L:D) h on agar-based lima bean diet in plastic 1 oz sample cups with snap on lids (SJVASC Insectary, 2008). They were delivered to Washington State University (Pullman, WA, USA) by FedEx overnight shipment. Wang et al. (2002) found that overnight air-shipment of codling moth did not affect the viability of any of the tested life stages. Eggs were supplied on waxed paper strips fastened with double stick tape to the bottom of plastic Petri dishes. Post-embryonic stages were treated in diet cups when they were 1 week (2nd to 3rd instar), 2 weeks (5th instar), and 3 weeks (pupae) old.

All life stages of codling moths were exposed to the LPLT environment maintained at 10 ± 0.5 °C, 1.33 ± 0.03 kPa pressure and near saturated relative humidity. The LPLT system included two identical hypobaric chambers. For chamber #1, all test insects to be treated were placed in the chamber which was then sealed and brought to the target pressure of 1.33 kPa. The chamber was opened and samples of each targeted life stage were removed after 6, 8, 10, and 12 days of treatment. After each sample was removed the chamber was re-sealed and brought back to 1.33 kPa. The entire process of opening the chamber to remove a sample took less than 30 min. To evaluate the effect on insect mortality of opening the chambers to take out samples, test insects in chamber #2 were held at 1.33 kPa continuously for 12 days. Additional samples were held at normal atmospheric pressure (NAP) and 10 °C for 12 days to evaluate the effect of cold storage alone. Untreated insects held at room temperature (25 °C) were used as controls. In addition, egg samples were held at 28 °C, 60% RH and photoperiod of 14:10 (L:D) h at SJVASC as non-transit laboratory controls.

Immediately after treatment, a small amount (<1 g) of wheat bran based insect diet (SJVASC Insectary, 2006) was added to the egg dishes to provide food and humidity for hatching larvae. Dishes were held at 25 °C for at least 10 days after treatment and then frozen to kill any hatched larvae before returning them to SJVASC for observations. Egg mortality was calculated based on the percentage of unhatched eggs. Post-embryonic stages after treatment were held at 25 °C and a photoperiod of 14:10 (L:D) h until adult emergence. The number of treated insects was determined by counting the number of adults emerging from untreated room temperature controls. For each treatment, three dishes each containing about 50 eggs and 21 diet cups containing 34–44 larvae or pupae were used. The test was replicated three times, and mean values and standard deviations of insect mortality were calculated.

Insect mortality values from all treatments were analyzed using the least significant difference (LSD) *t*-test. Mortality data from the 6, 8, 10 and 12 d exposures for the post-embryonic stages were analyzed using the probit procedure in PoloPlus 2.0 (Robertson et al., 2003). Lethal exposure times for 50 and 95% mortality (LT_{50} and LT_{95}) were estimated for each post-embryonic stage. Estimated exposure times were compared among all life stages by using the lethal-dose ratio test in PoloPlus 2.0 (Robertson et al., 2003, 2007).

2.3. Quality evaluation

Red Chief 'Red Delicious' apples were obtained from the Washington State University Turkey orchard (Pullman, WA, USA). The apples were harvested at the climacteric state and then held in a cold storage room at around 2 °C and 90–95% relative humidity before the LPLT treatment. Samples of 50 apples (about 8.5 kg) were exposed to a LPLT environment of 1.33 kPa, 10 °C, and a nearly saturated humidity level (>98%) for 6, 9, 12 and 15 days. Additional apples were kept at room temperature (25 °C) or in the cold storage room for comparison with the LPLT treatment. Weight loss, color, firmness, titratable acidity (TA) and soluble solids content (SSC) were selected as the quality parameters to evaluate the quality of apples after the LPLT treatment. All treatments were replicated three times.

The weight loss percentages were calculated based on the initial weight of the apples. The skin color of the 'Red Delicious' apples