THE MORTALITY OF ARTIFICIALLY INFESTED THIRD INSTAR LARVAE OF Anastrepha ludens AND A. obliqua IN MANGO FRUIT WITH INSECTICIDAL CONTROLLED ATMOSPHERES AT HIGH TEMPERATURES

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Abstract

Mango fruit in Mexico can be infested with several insects including various species of fruit flies. Hot water (46.1°C for 65-90 minutes, depending on fruit weight) is the only authorized quarantine treatment in the country. Some mango cultivars are tolerant to insecticidal atmospheres and to heat. Previous work in our laboratory and also reported in this meeting indicated that insecticidal controlled atmospheres (0-0.5 kPa O₂ + 50 kPa CO₂) at high temperatures (44-55°C) are very effective in causing in vitro mortality of eggs and third instar larvae of Anastrepha ludens and A. obliqua. Therefore in this work mango fruits were artificially infested with the third instar larvae of both species and larval mortality was determined after the fruits were exposed to the same atmosphere at 35-49°C and 50% RH for 160 min. Temperatures at 35-40°C caused 100% mortality of larvae of A. obliqua, but not of A. ludens. Temperatures at 42 to 49°C caused 100% mortality of the third instar larvae of both species.

1. Introduction

Mango fruits in Mexico can be infested with several insects, among which the most important one is the fruit flies Anastrepha ludens and A. obliqua. These are quarantine pests in several tropical and subtropical fruits. All mango fruits exported from Mexico to USA and Japan are treated with hot water at 46.1°C for 65, 75 or 90 minutes (depending on fruit weight). This treatment, especially for longer periods, accelerates ripening and causes fruit injury (Campos and Yahia, 1991). 'Keitt' mango is tolerant to insecticidal atmospheres (0.1-0.5 kPa O₂ and/or 50-80 kPa CO₂) for up to 5 days at 20°C (Yahia, 1998a; 1998b; 1994; 1993; Yahia et al., 1989; Yahia and Paull, 1997; Yahia and Tiznado-Hernandez, 1993; Yahia and Vazquez-Moreno, 1993). Several insects can be killed under these atmospheres (Paull and Armstrong, 1994). However, from a practical standpoint, a quarantine treatment should be accomplished in a shorter time. Insect mortality by modified (MA) and controlled atmospheres (CA) is faster at higher temperatures (Paull and Armstrong, 1994; Yahia, 1998a). Mango is relatively tolerant to heat, and thus quarantine treatments were developed based on the use of hot water or hot air (Heather et al., 1997; Paull and Armstrong, 1994). Mortality of the third instar larvae of A. ludens was significantly higher at 44°C in 1 kPa O₂ than in air (Shellie et al., 1997). Whiting and Van Den Heuvel (1995) have shown that CA significantly reduced the duration of exposure required for 100% mortality of Tetranychus urticae Koch.

There is no previous research reported on the effect of insecticidal CA at high temperatures on the in vivo mortality of the third instar larvae of A. ludens and A. obliqua. The objective of this work was to evaluate the effect of insecticidal CA (0-0.5 pKa O₂ + 50 kPa CO₂) at high temperatures (35-49°C) and 50% RH for 160 min.
2. Materials and methods

2.1. Insects handling and treatments
Third instar larvae of *A. ludens* and *A. obliqua* used in this experiment were obtained from the Moscafrut program in Chiapas. They were reared on artificial diet (Planta Moscafrut, 1996) at 27°C, transported to the laboratory in the same diet when they had 7 days of development from oviposition, and were used on the 8th day which corresponded to the third instar stage (Leyva-Vazquez, 1988).

2.2. Fruits
Physiologically mature mango fruits (*Mangifera indica* cv. Manila) were harvested after 90 days from fruit set in the Research Station Cotaclla, Veracruz. Upon arrival to the laboratory the fruits were selected for freedom of defects, uniformity, and were sorted to 24 lots (treatments) of 50 fruits each, and 14 lots (control) of 25 fruits each. Average fruit weight was 186.7 (±30.6). Fruits were left overnight at 20°C to ensure a homogeneous center temperature.

2.3. Artificial infestation
A cork borer (size 7, 1.5 cm of diameter) was used to bore 2 holes into the core of each fruit. The lower 1 cm of the plug was removed with a razor blade to leave sufficient space for the larvae, and 10 larvae were inserted into the center of each hole, with a total of 20 larvae in each fruit. The tissue plug was replaced and secured with low melting point hot glue. The control fruits were infested in the same way but were kept continuously at 20°C.

2.4. Heat and CA treatments
Treatments with CA at high temperatures were conducted inside a gas tight and temperature controlled chamber (Yahia et al., 1997). The chamber (155.8 cm high, 70.03 cm wide, and 132.08 cm deep) is constructed from stainless steel sheet metal. It accommodates 4 plastic boxes each with a dimension of 60.6 cm long, 14.4 cm wide, and 17.8 cm high, and a capacity of about 20-25 kg. Boxes are perforated in the bottom to enable vertical air flow. The control panel provides analog and digital input/output interfaces for an air velocity transmitter, O2 analyzer, CO2 analyzer, temperature probes, solenoid valves, fan, and heater elements. Auto/off/manual switches allow for manual operation of equipment for testing or backup control purposes. Monitoring and control functions are performed by a computer based control system. Operator control functions are accomplished through simple keyboard commands. The on/off status of components is indicated through graphical objects and/or textural messages. Vivid color graphic displays provide real time information in digital and trend graph formats. Historical data can be imported to a spreadsheet program for further analysis and data manipulation. All on/off control functions have auto/off/manual control switches provided for manual control. Audible and visual alarms are generated by the system when abnormal conditions occur. O2 and CO2 concentrations are directly controlled by injection of air, N2, and CO2 as required to maintain a desired set point. Control scheme uses time proportioned response to set point deviation in order to maintain parameters within 0.1% of the desired values. Deviation response can be individually tuned the chamber to achieve optimum control action. The O2 analyzer provides readings over a range of 0 to 25 kPa, and controlled within 0.1 kPa by injecting air/O2 or N2 as required. Two N2 solenoid valves provide two-stage control for stabilizing the O2 level after sealing the chamber. The CO2 analyzer provides readings over 0 and 80% and controlled within 0.1 kPa by injecting CO2 or N2 as required. Two carbon dioxide solenoid valves provide two-stage control to stabilize the CO2 level within one hour after sealing the chamber. O2 and CO2 analyzer performance is checked during each sampling cycle by comparing outputs against known standards, and any drift in calibration is automatically compensated for by the computer system. The chamber is automatically monitored for concentration of O2 and CO2. Readings are
displayed on graphic screens in bar graph, trend graph, and digital formats. Values are also recorded in historical files for archival record keeping, which can be easily formatted for analysis with a spreadsheet program. The N₂ and CO₂ selenoids are energized or de-energized based on the need for these gases in the chamber. If the level of O₂ rises above a set point, the system energizes the N₂ selenoid, allowing N₂ to flow into the chamber and to drive down the O₂ to the desired level. Each selenoid operates separately depending on demand. When N₂ is needed, as a first level of response, the system energizes the N₂ selenoid that leads to the humidification nozzles. If this does not provide enough N₂ to meet the demand, the second N₂ selenoid leading to the air duct is energized. The two CO₂ selenoids operate on the same principles. Air samples to be analyzed pass through the humidification nozzles, and at this point the air pressure regulator holds the pressure to 10 PSI. Another line tees off the compressor discharge, where a precision instrumentation regulator further reduces the pressure before the air continues to the flow meter located on the outside of the analyzer panel. The gas sample passes through the analyzer and back to the chamber. After the precision instrumentation regulator and before the analyzer, the air flow through a heat sink condenser, which cools the sample to approximately 27°C, a temperature acceptable to the analyzer. The heat sink condenser is a stainless steel tank filled with water. Additional moisture removal devices are installed before and after the analyzers to prevent the buildup of condensation. The O₂ analyzer is an electrochemical type, which sends a 4-to-20 milliamp output to the control system analog input module. This signal is converted to an O₂-level measurement of 0 to 25 kPa.

The CO₂ analyzer is an infrared that produces an output of 0 to 1 volts to an analog input module. The O₂ and CO₂ analyzers are calibrated routinely using the following calibration standards gases: 100% N₂ (Infra), 2.09% + 39.54% CO₂ + 58.37% N₂ (Aga), 4.99% O₂ + 5.1% CO₂ + 89.9% N₂ (Aga), and 9.95% O₂ + 9.98% CO₂ + 80.07% N₂ (Praxair).

Chamber temperature is elevated above the room temperature by means of an electric strip heater that is energized using time proportioned control technique, and temperature is maintained within ±0.1°C over the range of 20 to 60°C by automatically energizing four 1000W finned, 230V electric heater elements as required. These heater elements are automatically turned on by the control system. Temperature of the chamber is continuously monitored and displayed on graphic screens in digital and trend graph formats. Values are also recorded in historical files for archival keeping. Two air temperature thermistor probes measure the chamber supply air and discharge air temperatures. Twelve hypodermic thermistor probes monitor fruit surface or core temperatures as follows: surface low temperature, surface average temperature, surface maximum deviation, core temperature, core average temperature, and core maximum deviation. The probes measure 7 cm in length and 0.16 cm in width. Each lug has 3 probes which are connected to a 1/4-turn electrical connector with 2 pins (2 per thermistor). To create a signal that the computer can convert to a temperature reading, a Wheatstone bridge signal conditioning module provides excitation voltage across the thermistor, producing an output scaled from 0 to 100 millivolts. This output is connected to an analog input module which converts the millivolts to a temperature range of 20-60°C. The chamber uses a Wheatstone bridge signal conditioning module, creating an output signal for the temperature probes. The system sequentially reads each of the 14 probes, updating every 28 seconds. The air flow is generated by a 230V, single phase, 1.5hp centrifugal fan, located at the lower rear side of the unit, and has a cast aluminum fan blades. The fan discharges into a transition piece duct that distributes the air flow evenly into the bottom of the lowest lug. This is adjustable by using the horizontal and vertical fins located on the air register. Entering a duct at the top of the chamber above the top lug, the air passes through heater elements, blows through humidification nozzles, and returns to the fan. The return duct drops below the fan intake, creating a water reservoir or a drip leg. A stainless steel pipe drains any collected water to a drain on the right side below the control panel. Ports on the supply and return air ducts are connected to fittings on the right rear side of the unit. These fittings are used to connect to a manometer to determine the differential pressure (the difference between the air pressure in the supply...
duct and in the return duct). The differential pressure measures the resistance to air flow created by the fruit. Humidity is provided through four atomizing nozzles, each has two ports, one for compressed gas and one for water. When the compressed gas passes through the nozzle it draws distilled water from the humidification water reservoir into the nozzle using the Venturi effect. When more humidity is required, two selenoid valves turn on simultaneously, each allowing compressed air to flow through two nozzles. A back pressure regulator keeps the air pressure at a constant 10 PSI. When the valves open, the compressed air with atomized water flows into the chamber. The humidification water reservoir is manually filled. Two additional nozzles inject N₂, CO₂, and air along with atomized water. To control the flow of these gases, the system uses one air, two N₂, and two CO₂ selenoid valves. The air selenoid, one N₂ selenoid, and one CO₂ selenoid are teed together and linked to the humidifier nozzles. The other N₂ and CO₂ selenoids are teed together and are linked to the air duct, where large quantities of these gases can flow freely into the chamber without the restriction of the nozzles.

2.5. Mortality evaluation
Immediately after the treatments, fruits were cooled with ambient water for 30 minutes. Larvae were removed from the treated and non-treated infested mango fruits after 24 hours from treatment, placed in an incubator at 25-27°C and 60% RH and observed for 24 hrs. Larvae were scored daily as dead, alive, or pupated until they are either pupated or died. Non-treated infested mangoes were used to correct for handling and natural mortality according to the formula of Abbott (1925). Survived larvae were those that conserved their mobility. Dead larvae were deposited in distilled water and observed for several days to insure their death. Mortality was recorded as failure of the larvae or pupae to emerge as adults.

3. Results and discussion
A typical profile of changes in air (chambers supply and return air) and fruit (surface and pulp) temperatures, along with changes in O₂ and CO₂ concentrations during treatment at 46°C for 160 min are shown in figure 1. Depending on the type of treatment, supply air usually reaches desired temperature after about 10 min; return air reaches desired temperature after about 20 min. Fruit surface and pulp reach desired temperatures after about 80 to 100 min, pulp temperature being usually the slowest. Oxygen and CO₂ reach desired concentrations fairly rapidly; after about 10-20 min.

Temperature of 35-40°C caused 100% mortality of larvae of A. obliqua, but not of A. ludens (Table 1). The mortality of the third instar larvae of A. ludens at 35, 37 and 39°C was inconsistent. Slightly lower mortality was achieved at 35 and 39°C than at 37°C. This is probably due to the differences in the handling of the insects at origin, during transport, or during the experiment. A 100% mortality of the third instar larvae of A. ludens and A. Obliqua was achieved after exposure to ≥ 40°C (Table 1). Preliminary studies (Yahia et al., 1997) indicated that 44°C for 160 min caused 100% mortality of the third instar larvae of A. ludens, however, when exposure time was reduced to 120 min at 44°C in low O₂ and high CO₂, mortality was reduced to 62.6 and 71.5%, respectively. It was expected that the treatments required for 100% in vivo mortality would be more extreme than that for in vitro mortality. However, in vitro studies (Yahia and Ortega, 1999a) have shown that 100% mortality was achieved in a much severe conditions of temperature and length of treatment than what was achieved in vivo. For example in the in vitro studies (Yahia and Ortega, 1999a), 99.6% mortality of the third instar larvae of A. obliqua was achieved in CA (0 kPa O₂ + 50 kPa CO₂) at 46°C for 160 min, and 100% mortality of the third instar larvae of A. obliqua was achieved after exposure to CA at 48°C for 220 min. This was also observed by Whiting and van de Huevel (1995) and Lay-Yee and Whiting (1996) for Tetranychus urticae. This might be due to internal fruit factors that can increase the mortality of the larvae, or due to instability problems of the insect inside the fruit. Insect response to anoxia and hipoxia is not well understood.
Soderstrom et al. (1990) found that low \( \text{O}_2 \) (0.5 kPa) is less effective than high concentration of \( \text{CO}_2 \) for the mortality of codling moth at 25°C. However, Shellie et al. (1997) found that reduced \( \text{O}_2 \) concentration (1 kPa) was more lethal to \( A. \text{ ludens} \) larvae than was an enriched \( \text{CO}_2 \) atmosphere (20 kPa).

## Conclusions

We conclude that CA (0 kPa \( \text{O}_2 \) + 50 kPa \( \text{CO}_2 \)) at \( \geq 40^\circ \text{C} \) for 160 min is effective in controlling (100% mortality) the third instar larvae of \( A. \text{ ludens} \) and \( A. \text{ obliqua} \) artificially infested in mango fruit. However, the efficiency of this treatment needs to be confirmed using higher number of insects and fruits. In case that these results are confirmed, these treatments can be applied as quarantine systems for \( A. \text{ ludens} \) and \( A. \text{ obliqua} \). Yahia et al. (1997) and Yahia and Ortega (1999b) have shown that mango fruit tolerate these CA treatments for temperatures up to 43°C for 160 min.

## References


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Conclusions

We conclude that CA (0 kPa O₂ + 50 kPa CO₂) at ≥ 40°C for 160 min is effective in controlling (100% mortality) the third instar larvae of A. ludens and A. obliqua artificially infested in mango fruit. However, the efficiency of this treatment needs to be confirmed using higher number of insects and fruits. In case that these results are confirmed, these treatments can be applied as quarantine systems for A. ludens and A. obliqua. Yahia et al. (1997) and Yahia and Ortega (1999b) have shown that mango fruit tolerate these CA treatments for temperatures up to 43°C for 160 min.

References


Table 1 Average corrected mortality of third instar larvae of Anastrepha ludens and A. obliqua artificially infested in 'Manila' mangoes and exposed to CA (0 kPa O$_2$ + 50 kPa CO$_2$) at high temperatures and 50% RH

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<tr>
<th>Temperature (°C)</th>
<th>Average corrected mortality (%)</th>
<th>A. ludens</th>
<th>A. obliqua</th>
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<td>35</td>
<td>99.18 (± 0.83)</td>
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<td>39</td>
<td>98.85 (± 1.05)</td>
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Values in parenthesis indicate standard error of the mean.
Figure 1  A profile of changes (entrance and exit air), and fruit (surface and pulp) temperatures, and \( O_2 \) and \( CO_2 \) concentrations during treatment in CA (0 kPa \( O_2 + 50 \) kPa \( CO_2 \)) at 46\(^\circ\)C and 50\% RH for 160 min.