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VACUUM AS A METHYL BROMIDE ALTERNATIVE FOR DISINFESTATION OF DURABLE AND FRESH COMMODITIES

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ABSTRACT

Low pressure, or vacuum, can kill insects by achieving a low oxygen atmosphere. Previous work with life stages of several stored product insect species showed that a vacuum of 50 mm Hg, equivalent to 1-2% oxygen, can kill tolerant eggs and pupae between 12-200 h at temperatures from 37°C down to 5°C. This work investigated the possibility of treating pests of fresh commodities with vacuum, and also tested the potential for using a combination of vacuum with another toxicant to shorten treatment time. Tests with eggs of the apple maggot fly, *Rhagoletis pomonella*, infesting apples found that 99% mortality was achieved at 58 h (30°C) or 104 h (25°C) at 50 mm Hg. Market-quality apples of Red Delicious and Golden Delicious varieties were subjected to a 50 mm Hg vacuum treatment for 5 days at 25°C with no significant loss of market quality, as determined by a human assessment panel. Practical and low-cost application of vacuum to bagged or bulk commodities has been proposed using flexible PVC Cocoons, but innovations in structural support will be needed to prevent damage of crushable products from the strong external forces surrounding an evacuated Cocoon. Experiments with eggs of the Indianmeal moth, *Plodia interpunctella*, and the lesser grain borer, *Rhyzopertha dominica*, found that addition of the natural product fumigant ethyl formate to a vacuum treatment of 50 mm Hg reduced treatment times for these pests to less than 4 h at 25°C. Thus, potential exists for vacuum-based treatments of commodities that are as effective and as time-efficient as those with methyl bromide.

INTRODUCTION

The search for alternatives to methyl bromide (MB), a broad-spectrum fumigant for treatment of perishable and durable commodities and a known ozone-depleting substance, has motivated research into chemical and non-chemical treatments that are environmentally friendly, non-residual and fast-acting. The Montreal Protocol of 1995 mandates a phase-out and eventual elimination of production and use of MB in developed and developing countries by 2005 and 2020, respectively (United Nations Environment Programme 1998). Numerous chemical and non-chemical alternatives have been investigated and proposed, but each has limitations in being effective over a broad-spectrum use like methyl bromide (Bell *et al*. 1996).

Use of low pressure is one of the potential alternatives for disinfestation of durable and perishable commodities. Research into use of low pressure for disinfestation of stored-products infested by storage insects dates back more than 75 years (Back and Cotton 1925) followed by investigations by several researchers (Bare 1948, Calderon *et. al*. 1966, Calderon and Navarro 1968, Navarro and Calderon 1979, Freidlander and Navarro 1983, Mbata and Phillips 2001, 2004). Low pressure application to a commodity creates low-oxygen atmosphere that kills insects; the low oxygen effect apparently predominates over other physical effects of low pressure, such as structural breach of cell membranes or dehydration (Navarro and Calderon 1979, Friedlander and Navarro 1983). Most of the research on use of low pressure has been done for disinfestation of stored-product insects and their immature stages in durable commodities. We did not find any research to investigate the effect of low pressure in controlling insect-pests of perishable commodities like fruits. Combinations of postharvest treatments for insect disinfestations have been reported in the past, and recently vacuum was combined with propylene oxide in laboratory trials (Navarro *et al.* 2004).

The present work had two main objectives. First, we sought to investigate the effect of vacuum on survival of eggs and larvae of the apple maggot fly, *Rhagoletis pomonella*, (Walsh) (Diptera: Tephritidae), in apples at two pressures (25 and 50 mmHg) and temperatures (25 and 30°C) for various exposure times, and to additionally determine the effects of vacuum treatment on apple quality. Second, we conducted a preliminary investigation into the effect of combining low pressure treatment with addition of the fumigant ethyl formate with the aim of developing a 4-h treatment to control stored-product insects.

MATERIALS AND METHODS

Infestation of Apples with Apple Maggot Flies.

Red delicious' apples were infested with apple maggot (AM) flies by exposing them to adults in cages (11" x 11", 12" high) at 27°C, 65% RH and for a period of 8 h. Experiments were conducted at two pressures, 25 and 50 mmHg, and at two temperatures, 25 and 30°C. Egg-mortality experiments were conducted within 24 h of infestation by adult AMs; larval-mortality tests were conducted on apples held at 25°C for 11 days after oviposition by adult AMs.

Low Pressure Treatment of Apples.

Apples infested with AM were placed in 3.8 l glass jars (25 cm tall by 15 cm dia, with neck diameter of 11.4 cm) on a 15 mm thick layer of white sand. Out of each batch of AM infested apples (24 to 30 apples in a batch) available on a given day, three or four apples were placed inside each of several treatment jars. The screw-on metal lid of each glass jar was made gas-tight by addition of a rubber O-ring and use of vacuum grease around the jar lip. Each jar lid was equipped with an evacuation port that had a ball-type shut-off valve, a flexible vacuum hose that was connected to a pump, and a dial-type pressure gauge to measure low pressure in a range of 0 to -760 mmHg. We used a laboratory vacuum pump (Duo-Seal Co., Chicago, IL) to obtain the desired experimental pressures in each glass jar. Upon reaching the target pressure the ball-valve on the treatment jar was closed, the vacuum hose was disconnected from the pump, and the glass jar was transferred to a temperature controlled chamber and maintained at either 25 or 30°C for various exposure times. Six or more exposure times were used for the trials at a combination of pressure and temperature and ranged from 1 to 120 h. Ventilated plastic containers (21.6 cm by 17.8 cm) with a layer of white sand at bottom (15 mm thick) were used to store the individual treated apples to allow for emergence of next developmental stage following treatment. Untreated control apples were handled the same way as low-pressure treated apples, except that they were maintained under ambient pressure at all times in ventilated plastic holding containers. The number of treated flies per group of apples was estimated from the number of pupae that emerged from similar groups of untreated apples that were set up at the same time. Data on estimated number of AMs killed by a given treatment were subjected to probit analysis (SAS Institute 2000), and comparison among treatments and between life stages were done using the lethal dose ratio test (Robertson and Preisler 1992).

Quality Treatments and Sensory Evaluation of Treated Apples.

Batches of market quality ‘Red Delicious’ and ‘Golden Delicious’ apples were procured from local markets and divided into control and experimental groups. The experimental groups were treated at 50 mmHg at either 25 or 30°C for a period of 3 or 5 days, based on results of the insect mortality experiments. A replicate for a cultivar trial at a designated temperature and pressure consisted of 3 apples in a vacuum jar for each exposure time. There was a total of three replicates for each of the two exposure times. Hence, in a trial with one cultivar and temperature, there were six vacuum desiccators under low pressure (50 mmHg) treatment, three for the 3-day treatment and three for the 5-day treatment. At the end of exposure periods the apples were taken out and stored in paper bags at 1°C for a period of 14 days. Untreated controls were stored in paper bags in cold storage at 1°C and ambient pressure for 19 days. Following the cold storage, the treated and untreated apples were taken out and kept at room temperature (25°C) for 6 h prior to evaluation by a human sensory panel of 9 people. Each panelist evaluated three numbered apples of a given cultivar, one from each treatment group and the control group, for external and internal appearance, and taste. Evaluation for each of the three criteria was done by rating on a numerical scale of 1 to 5, with 5 being rated as best for all three criteria.

Studies Combining Vacuum with Ethyl Formate.

Eggs of *Plodia interpunctella*, the Indianmeal moth, and *Rhyzopertha dominica*, the lesser grain borer, were collected from laboratory colonies of insects and used within 24 h of oviposition. Groups of 20 eggs were placed on sticky cards in ventilated glass vials, together with approximately 0.5 g of ground wheat, and subjected to treatments in the same vacuum jars as described above. Jars lids were modified for these studies by addition of a T-junction and an injection port with rubber septum through which liquid ethyl formate could be added using a syringe. Synthetic ethyl formate was obtained from Aldrich Chemical Co. (Milwaukee, WI),and it volatilized quickly at room temperature upon injection into jars. All trials utilized a four-hour treatment period at 25°C in order to simulate the temperature and time constraints of a typical methyl bromide treatment. Experiments with vacuum and ethyl formate each applied separately were initially conducted to determine if these could be effective alone. Combination treatments were ultimately done at 50 mm Hg with varying doses of ethyl formate. Volumes of ethyl formate were injected into jars to deliver concentrations up to 200 g/m^3. Untreated controls were simply held at 25°C under ambient pressure for 4 h. Following treatments the eggs were held at 30°C at ambient pressure in fresh air for up to seven days, and then the number of unhatched eggs were determined and designated as killed.

RESULTS AND DISCUSSION

Low pressure treatment of *R. pomonella* infested apples.

Table 1 reports the predicted LT_{99} levels for eggs and larvae of *R. pomonella* at two temperatures and two pressures resulting from exposure times ranging from 3 to 120 h. The eggs were more tolerant compared to larvae in all cases at both pressures and temperatures, and this result is similar to what has been found for stored product insects (Mbata and Phillips 2001). Complete mortality of eggs was achieved in 106 and 98 h at 25°C and low pressures of 25 and 50 mmHg, respectively. At 30°C, 100% mortality of eggs was achieved in approximately half the time compared to 25°C demonstrating the effect of higher temperature to achieve rapid mortality. Since the time required to achieve 99% mortality of eggs was statistically similar at either 25 or 50 mm Hg of pressure (Table 1), we recommend that a pressure of 50 mm Hg be adopted for commercial application because this pressure is easier to reach and maintain with a commercial vacuum pump than a lower pressure like 25 mm Hg. Studies on fruit quality thus utilized 50 mm Hg of pressure.

Consumer evaluation of low pressure treated apples.

Low pressure treatment, whether at 25 or 30°C, had no effect on the rating of 'Golden delicious' apples by human panelists for all three quality parameters scored. Panelists reported that, on average, untreated Golden Delicious apples, and those treated at 50 mm Hg at either 25 or 30°C for 3 or 5 days were rated on a 5-point scale at 3.0-3.89 for external appearance, 3.67 –4.33 for internal appearance, and 3.22-3.89 for taste, and there were no statistically significant differences among any of the treatment groups for any quality parameter. However, in the case of the 'Red delicious' cultivar, panelists reported that internal and external appearances were significantly affected by treatments, but taste was not (Fig. 1). 'Red delicious' apples treated at 30°C had significantly lower panel ratings for external and internal appearance than untreated control apples (Fig. 1b); whereas at 25°C the rating for external and internal appearances were slightly improved by the 5-day vacuum treatment compared to others (Fig. 1a). Taste was apparently unaffected by any treatment. Reason for lower panel ratings of 'Red Delicious' apples treated at 30°C was likely due to a darker appearance to the red skin of some treated apples, and observations of darkened areas of the internal flesh in some cases. External appearance is one of the important factors for marketability of fresh fruits and therefore low pressure treatment at 30°C may not be proper choice for 'Red delicious' cultivar, but treatments at 25°C or lower may be appropriate. The results show that cultivar response to low pressure varies at different temperatures. This work should be continued and expanded to determine the utility of vacuum for treating fresh commodities in various circumstances. Ultimately, commercial application of vacuum to damageable products such as fresh agricultural commodities will a require a chamber that can deliver the required conditions. Use of flexible "cocoons" for vacuum treatment chambers (Villers 2001) would require a structural addition to prevent damage of the product from the high external loads of the cocoon imposed by the low pressure of the system.

TABLE 1

Exposure time (h) and fiducial limits (with 95% CI) required to obtain 99% mortality (LT_{99}) of eggs and larvae of apple maggot flies (*R. pomonella*) at two temperatures and pressures following probit analyses.

Pressure	Life Stage	Temperature, °C	
(in mm)		25	30
25	Eggs	105.98 Aa (99.89 - 113.01)	51.46 Ba (48.54 - 54.90)
	Larvae	10.16 Ac (9.88 - 10.43)	20.97 Bc (19.12 - 23.30)
50	Eggs	97.55 Aa (91.78 - 104.32)	52.48 Ba (48.08 - 58.04)
	Larvae	63.62 Ab (59.14 - 69.00)	33.411 Bb (30.53 - 37.04)

Combination Treatments of Vacuum with Ethyl Formate.

When three different low pressures were applied to eggs of either Indianmeal moths or lesser grain borers we found that mortality (non-hatch of eggs) was very low after a four-hour exposure period (Table 2). Indianmeal moth eggs exposed to vapors of ethyl formate only were killed at a level of 88% at a dose of 150 gm/m^3, while lesser grain borer eggs had 100% mortality when exposed to 200 gm/m^3 (Table 3). For both species there was a decrease in the amount of ethyl formate needed for 100% kill when this was combined with a vacuum of 50 mm Hg. This effect was pronounced for Indianmeal moths, which experienced 100% mortality upon exposure to just 8 gm/m^3 ethyl formate combined with vacuum (Table 4). The combination treatment also enhanced mortality of lesser grain borer eggs, which had 100% mortality after exposure to 50 gm/m^3 of ethyl formate combined with vacuum (Table 5).

TABLE 2
Mortality of eggs of two species of stored product insects exposed to low pressure only for 4 h at 25°C

mm Hg	Mean % Mortality	
	Indianmeal Moth	Lesser Grain Borer
Ambient (control)	2.0	3.5
100 mm	1.0	5.5
50 mm	7.0	6.5
25 mm	10.0	8.5

TABLE 3
Mortality of eggs of two species of stored product insects exposed to doses of ethyl formate only for 4 h at 25°C

gm/m^3	Mean % Mortality	
	Indianmeal Moth	Lesser Grain Borer
0 (control	9	3
10	48	24
50	70	90
100	81	99
150	88	(not tested)
200	(not tested)	100

TABLE 4
Mortality of Indianmeal moth eggs treated with vacuum and ethyl formate for 4 h at 25°C

Vacuum (mm Hg)	Ethyl Formate (gm/m^3)	% Mortality
0 (control)	0	5
50	2	9
50	4	50
50	6	86
50	8	100

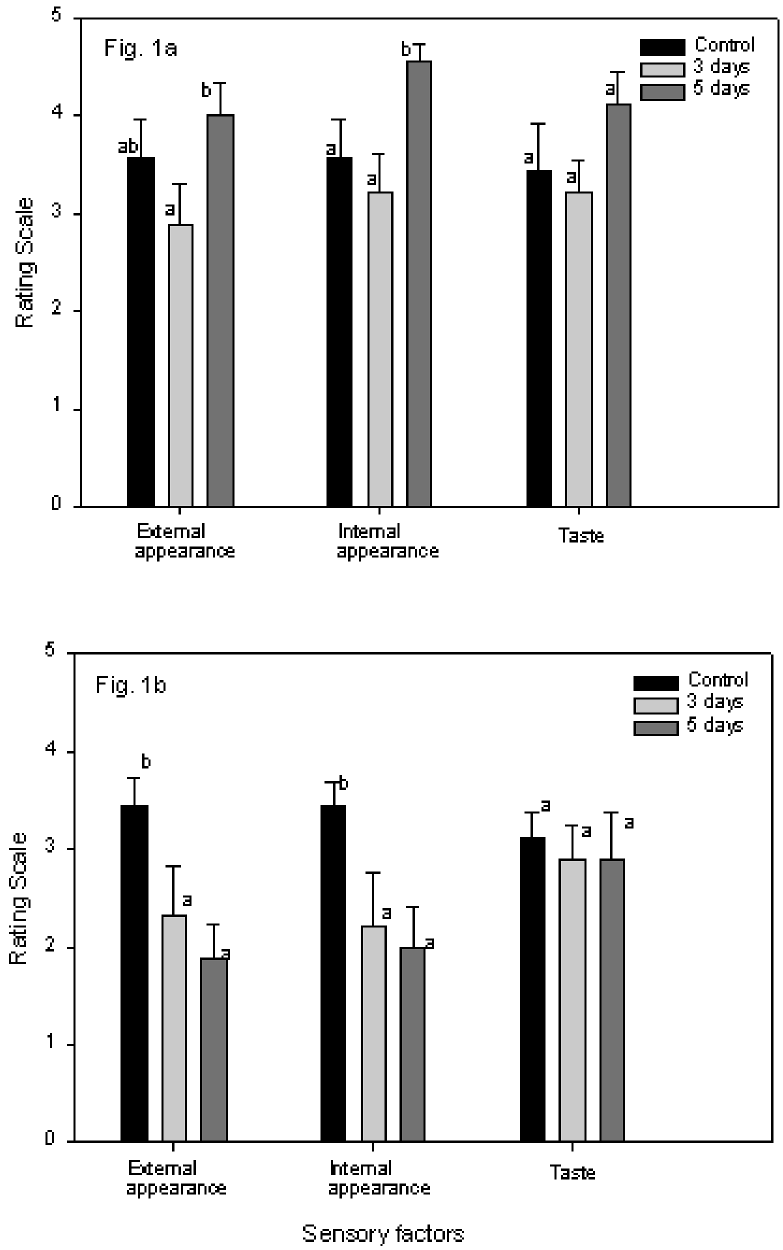

Figure 1. Effect on rating of external and internal appearance and taste of red delicious apples exposed to 50mm Hg at 25ºC (Fig. 1a) and 30ºC (Fig 1b) for 3 and 5 days.

CONCLUSIONS

There are two important outcomes in the work reported here. First, low pressure alone can kill tolerant life stages of internal infesting insects such as the apple maggot fly in fresh commodities like apples, and our results with consumer acceptability suggest that vacuum can deliver a treatment with minimal negative effects on product quality. Secondly, results from studies with ethyl formate and vacuum suggest that a combination of these two treatments can result in an improved effect compared to results with each treatment alone, and combination treatment may allow for a relatively short treatment time that is comparable to the times for methyl bromide treatments. Although ethyl formate is a chemical toxicant, it can be considered a reduced risk material because it occurs naturally in various foods and it is considered safe as a food additive (Damczevski and Annis 2001

TABLE 5

Mortality of lesser grain borer eggs treated with vacuum and ethyl formate for f4 h at 25°C

Vacuum (mm Hg)	Ethyl Formate (gm/m^3)	% Mortality
0 (control	0	5
50	10	21
50	20	54
50	30	91
50	50	100

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