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Optimal Low-pressure Conditions for Long-term Storage of Fresh Commodities Kill Caribbean Fruit Fly Eggs and Larvae

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SUMMARY. U.S. regulations prevent importation of fresh horticultural commodities that have not received an approved quarantine treatment assuring 99.999% (Probit 9) mortality of potentially invasive insect pests. Because imported mangoes (*Mangifera indica*) are likely to be infested by the caribbean fruit fly (*Anastrepha suspensa*) and other tropical fruit flies in the Americas, such as the mexican fruit fly (*A. ludens*), guava fruit fly (*A. striata*), inga fruit fly (*A. distincta*), south american fruit fly (*A. fraterculus*), sapote fruit fly (*A. serpentina*), and the west indian fruit fly (*A. obliqua*), they must be hot-water treated prior to shipment in order to satisfy quarantine requirement. Hot water treatment often damages the fruit, especially if it is not fully mature. Hypobaric [low pressure (LP)] intermodal shipping containers developed by the VacuFresh Corp. preserve fresh commodities, such as horticulturally mature mangoes, far longer than is possible using other technologies. We tested the ability of caribbean fruit fly eggs and larvae to survive simulated optimal hypobaric conditions for shipment of mangoes [15 and 20 mm mercury (Hg), ≥98% relative humidity, 13 °C (the lowest, safe nonchilling temperature)]. Caribbean fruit fly eggs or larvae were maintained on agar media, flushed with one air change per hour at the storage pressure, and shielded with Mylar to prevent radiant heat uptake and limit evaporative cooling. Nearly 98% of the eggs and larvae were killed within 1 week at 15 and 20 mm Hg in nine replicated experiments. All eggs were killed by 11 days with a predicted kill of 99.999% of the eggs by 9.4 days in 15 mm Hg and 10.6 days in 20 mm Hg LP (based on Probit 9 statistical analysis), whereas a substantial number of eggs survived to 14 days at atmospheric pressure (760 mm Hg). Shipment of fresh produce using this technology promises to provide quarantine control while preserving the freshness of fully mature tropical fruits and vegetables.

Importation of mangoes and other tropical fruits into the U.S. has steadily increased from Mexico, Central America, South America, and the Caribbean islands. U.S. regulations prevent importation of horticultural commodities that have not received an approved quarantine treatment assuring Probit 9 level (i.e., 99.999% mortality of potentially invasive insect pests). Numerous fruit fly species that are potential threats to U.S. agriculture infest tropical areas where mangoes and other tropical fruits are grown. Among the most economically important are members of the genus *Anastrepha*, including caribbean fruit fly, mexican fruit fly, guava fruit fly, inga fruit fly, south american fruit fly, sapote fruit fly, and west indian fruit fly (Aluja and Liedo, 1990). Adult flies oviposit eggs beneath the skin of immature fruit, producing larvae that destroy the pulp as mangos ripen. Because imported mangoes are likely to be exposed to these pests, they must undergo a U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA/APHIS)-approved hot-water quarantine treatment prior to packing in order to disinfest fruit of the various fruit fly species (Sharp, 1986; Sharp et al., 1988, 1989a, 1989b).

Growers commonly harvest fruit before horticultural maturity in order to extend the storage life during long-distance shipment in refrigerated intermodal containers. This practice results in poor eating quality for consumers and manifestation of physiological disorders that have led to rejection of shipments when immature fruit are heat treated to comply with current quarantine regulations. The added cost for equipment, maintenance, and USDA/APHIS personnel to oversee the hot-water treatments has caused some packers to forgo the treatment and ship their fruit to Europe, where quarantine treatments are not required. Moreover, heat treatment is certified as

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
3.7854	gal	L	0.2642
2.5400	inch(es)	cm	0.3937
25.4000	inch(es)	mm	0.0394
6.4516	inch ²	cm ²	0.1550
0.4536	lb	kg	2.2046
0.1333	mm Hg	kPa	7.5006
28.3495	oz	g	0.0353
(°F - 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

a means to disinfest mangoes weighing up to only 750 g, and many growers desire to ship larger fruit. Growers producing 'Keitt' mango fruit are particularly hampered by these constraints due to the large size of this popular cultivar.

Refrigerated low-pressure intermodal cargo containers can revolutionize the long-distance shipping of fresh, horticulturally mature fruits and vegetables that have a short storage life (Burg, 2004). Low oxygen (O₂) partial pressure and creation of a nearly ethylene- and carbon dioxide (CO₂)-free atmosphere inside and around the stored product prevent bacterial and mold growth and allow mature mangoes and other tropical fruits to be maintained for months without risk of ripening or loss of "tree ripe" quality (Alvarez, 1979, 1980; Apelbaum and Barkai-Golan, 1977; Borecka, 1986; Burg, 1975, 2004; Chau and Alvarez, 1983; Dilley, 1977; Wells, 1974). Cold-tolerant commodities, such as strawberries (*Fragaria × ananassa*) (Jamieson, 1980b), mushrooms (unspecified species) and asparagus (*Asparagus officinalis*) (Dilley, 1977), sweet cherries (*Prunus avium*) (Borecka, 1986), most types of flowers, cuttings, and potted plants (Burg, 1973, 2004), and numerous types of vegetables (Burg, 1990, 2004) are best stored in LP at pressures of 10 to 15 mm Hg. All fruits stored in LP conditions ripen normally 3 to 4 d after transfer to air at 25 °C with excellent eating quality (Burg, 2004; Spalding and Reeder, 1976).

This capability eliminates the need to harvest immature fruit in order to maintain quality during extended transits in conventional refrigerated containers operated at ambient, standard atmospheric pressure (760 mm Hg). The VacuFresh LP container (Welfit Oddy, Capetown, South Africa), fabricated by one of the world's leading tank container manufacturers, Welfit Oddy (2003), is a lightweight, energy-efficient unit capable of reliably and accurately regulating the O₂ partial pressure low enough to kill insects while ensuring fresh commodity quality.

Back and Cotton (1925) first reported killing various developmental stages of stored-product insects by LP, and Bare (1948) found that mortality resulted when tobacco moths (*Ephistia elutella*) and cigarette beetles (*Lasioderma serricorne*) were exposed to LP in a chamber containing the insects and

tobacco (*Nicotiana tabacum*) together. Numerous others have since reported high insect mortality in LP (Calderon and Leesch, 1983; Calderon and Navarro, 1968; Calderon et al., 1966; Cline and Highland, 1987; Navarro et al., 2001; Thorton and Sullivan, 1964; Villers, 2001; Yi et al., 1992). Viability of six species of common stored-product insects at 18 or 25 °C and pressures between 10 and 20 mm Hg was examined in containers filled with a wheat (*Triticum aestivum*) load (Calderon et al., 1966). The low pressures caused 100% mortality of larval and adult forms of the almond moth (*Ephestia cautella*), saw-toothed grain beetle (*Oryzaephilus surinamensis*), rust-red flour beetle (*Trilobium castaneum*), cowpea weevil (*Callosobruchus maculatus*), rice weevil (*Sitophilus oryzae*), and khapra beetle (*Trigoderma granarium*) within 120 h at both 18 and 25 °C. Adults of the almond moth, saw-toothed grain beetle, and cowpea weevil, and adults and larvae of the rust-red flour beetle were killed within 7 h. Within the 50- to 300-mm Hg range coupled with temperatures varying from 5 to 37.5 °C, the mortality of eggs of four common species of stored-product insects, indian meal moth (*Plodia interpunctella*), lesser grain borer (*Rhyzopertha dominica*), almond moth (*Cadra cautella*), and rust-red flour beetle, increased with greater exposure to conditions of lower pressures at a given temperature, or higher temperatures at a given pressure (Mbata et al., 2004).

An LP treatment at 15 to 20 mm Hg was 100% lethal to the green peach aphid (*Myzus persicae*) within 52 h (Aharoni et al., 1986), and a LP treatment of 50 mbar (37.5 mm Hg) was 96% effective in killing the lettuce aphid (*Nasonovia ribis-nigre*) and potato aphid (*Macrosiphum euphorbiae*) and the serpentine leaf miner (*Liriomyza langei*) infesting lettuce (*Lactuca sativa*) by 4 d (Liu, 2003). Mortality of the aphid was pressure and time dependent.

Insect mortality in LP appears to be a direct result of hypoxia (Fridlander and Navarro, 1983; Navarro and Calderon, 1979). The adult and larval stages of stored-product insects are the most susceptible, whereas eggs require longer periods of exposure to kill them (Finkelman et al., 2003; Mbata and Phillips, 2001).

There has been no published re-

search on the impact of LP on the larvae of fruit fly species that commonly infest mangoes and other tropical fruits. The purpose of the present research was to determine if the optimal mango storage condition in a refrigerated VacuFresh intermodal container reliably kills all caribbean fruit fly eggs and larvae during a minimal transit time. This species is common throughout tropical America and is typical of several fruit fly types of quarantine concern in the U.S. and Caribbean basin. If fruit flies can be killed in-transit, use of such a shipping container would eliminate the need for costly hot-water quarantine treatment for the fruit flies.

Materials and methods

LOW PRESSURE AND AMBIENT PRESSURE SYSTEMS. A lab-scale vacuum (LP) system was constructed that was capable of creating the temperature, relative humidity (RH), pressure, and airflow conditions that are optimal for shipment of tropical fruits such as mango in a VacuFresh container. The system consisted of three Nalgene 20-L, polycarbonate vacuum desiccators (Nalge Nunc International, Rochester, N.Y.) housed in each of two Conviron model PGW36 environmentally controlled rooms (Conviron, Winnipeg, Man., Canada) within which the temperatures were maintained at 13 ± 0.5 °C. Two of the desiccators in each room were used for LP storage and the third provided standard atmospheric pressure (760 mm Hg) comparison (AP) simulating a standard, refrigerated intermodal container. Each LP desiccator was supplied with a flow of one volume per hour to maintain ≥98% RH rarified air regulated with a Gilmont micro flow meter (BC Group International, St. Louis) operated with its flow control valve at atmospheric pressure. Humidified air was drawn through the AP chambers at a controlled rate of one air exchange per hour using an Emerson lab pump (Emerson Motor Division, St. Louis), separate from the LP system, coupled to a bubbling flask.

An absolute vacuum of 20 ± 0.5 mm Hg in the initial set of experiments and 15 ± 0.5 mm Hg in later experiments was provided to the LP desiccators by a gas-ballasted two-stage Welsh vacuum pump (HyVac Products, Royersford, Pa.). Pressure was regulated by a two-stage system comprising an in-line Fisher model Y695VR vacuum

regulator (Fisher Regulators, McKinney, Texas) coupled with a Moore Nullmatic sub-atmospheric regulator (model 43-20; Moore Products Co., Spring House, Pa.). Dry and wet bulb temperatures inside the desiccators were continuously monitored during each experimental run using Omega precision Teflon-coated thermistors (model 44104; OMEGA Engineering, Manchester, U.K.) inside the desiccators, coupled to an Omega HH40 series handheld thermistor thermometer unit to determine temperature and RH.

FRUIT FLY MEDIA AND REARING. Caribbean fruit fly eggs and larvae were obtained from the USDA fruit fly-rearing lab at the Subtropical Horticulture Research Station, Miami, according to established protocols. Fresh eggs oviposited by gravid female fruit flies were harvested by rinsing them from wax-coated cheesecloth membranes located on the sides of cages containing female and male flies. Eggs collected from several such cages were gently cleaned by rinsing them through a pre-filter to remove larger debris and subsequently caught in a 0.177-mm filter. They were then briefly rinsed with 0.26% sodium hypochlorite, rinsed again with water, and evenly suspended in 1% aqueous Cell-U-Wett gel (Griffin Corp., Valdosta, Ga.). The egg-laden gel was evenly spread, using a 10-mL serological pipette, down 1-inch-wide strips of Chix 0930 Masslinn cloth (Chicopee, Mooresville, N.C.) that had been premoistened with a 0.1% sodium benzoate solution. The strips were placed egg-side up on damp sponges to absorb extra moisture and maintain humidity and incubated in covered dishes overnight at 25 °C. For experiments involving 20 mm Hg, each of the egg-laden strips was cut into about 1-inch² pieces the following day, and each of these was placed egg-side down onto the media surface of each dish to facilitate development of a synchronized population of larvae in all dishes for each run of the experiment.

Nutrient agar medium was prepared by first stirring 124 g of agar into 16.8 L of hot distilled water until completely dissolved. Once removed from the heat, another 16.8 L water was added along with 41 g *p*-hydroxybenzoic acid, 41 g sodium benzoate, 20.5 g cholesterol, 2.46 kg glucose, 2.46 kg wheat germ, 2.46 kg torula yeast (*Torula* sp.), and 174 mL concentrated

hydrochloric acid. The mixture was stirred for about 10 min and dispensed into about 80 petri dishes with lids prior to gelling. These were then used in each experimental run.

We observed great variability in the number of harvested eggs per dish, ranging from 100 to 600 from one experiment to the next, in the 20 mm Hg tests. For the second phase of study examining the effects of 15 mm Hg on eggs, efforts were made to stabilize the egg numbers to about 300 eggs per dish. After the chlorinated rinse, the eggs were allowed to settle in a 100-mL graduated cylinder. The upper water portion was removed, and the eggs were dispensed onto Masslinn cloth, where the excess liquid was drained on a porous platform. Aliquots of eggs were transferred to tared weighing dishes, weighed, and suspended in 100 mL of Cel-u-Wett gel. The total number of eggs was estimated by counting the number of eggs in 1-mL spread on a petri plate with a marked grid and counted under a dissecting scope. It was determined that there were approximately 20,000 moist eggs per gram. In subsequent experiments, 1.5 g of moist eggs per 100 mL gel, providing about 300 eggs/mL, were used. A volume of 0.9 mL of egg suspension was sufficient to fill the space available on each 1-inch square of Masslinn premoistened with 0.1% sodium benzoate solution. They were incubated overnight at 25 °C, and placed egg-side down on the nutrient agar medium of each dish in all subsequent experimental runs. Despite these efforts, there was still substantial variation in initial egg hatches of nontreated controls, as evidenced by the number of larvae found in each dish, perhaps due to mechanical damage to eggs by the magnetic stir bar used to keep eggs suspended in the gel.

EGG EXPERIMENTAL PROTOCOLS. Groups of eight replicate covered petri dishes containing freshly placed eggs were loosely wrapped in Mylar reflective film to prevent evaporation of water in the medium in response to radiant heat transfer from the walls of the LP desiccator to the insects through the rarified air. These were placed in each of the four LP desiccators, and the two AP desiccators in the 13 °C cold rooms.

To determine the initial viable egg count and nontreatment survival rate, eight replicate dishes were individually

placed without lids in separate shallow bowls with mesh covers to prevent larvae escape. The dishes, henceforth called pupation bowls, contained a 1-cm-deep bed of No. 4 fine vermiculite (W.R. Grace, Columbia, Md.). They were incubated at 29 °C and high humidity for about 1 week to facilitate larval development and pupation. These served as “0 time” nontreated controls for both the LP- and AP-treated eggs.

To assess mortality of eggs after exposure to various durations in the LP and AP treatments, one group of eight dishes was transferred without lids from each of the LP and AP desiccators to pupation bowls in the 29 °C incubation cabinet after 4, 7, 11, and 14 d (4, 7, 9, and 11 d in later experimental runs). All pupation bowls were maintained until all surviving larvae had pupated, at which time the number of pupae in the vermiculite and agar media were counted.

LARVAE EXPERIMENTAL PROTOCOLS. At least 5 g of eggs were collected and transferred to the surface of nutrient agar media in two 35 × 25 × 5-cm trays and incubated at 25 °C until first and/or second instar larvae were active. The media was rinsed through a large sieve, and the retained larvae were transferred to a 20-L container of water and suspended by bubbling air in the water. A filter was constructed that was calibrated to catch about 300 larvae, which were then transferred to individual petri dishes containing media for incubation in the LP and AP treatments at 13 °C. In the first experiment comparing survival of first and second instar larvae, groups of 10 petri dishes containing each of the larval types were covered in Mylar film and treated in the AP and 15 mm Hg LP conditions for 1, 2, and 3 d before being transferred to pupation dishes in a 25 °C incubator. Ten plates of each larval type were also placed in pupation dishes in the incubator at 0 time to determine untreated survival rates of each larval stage. All larvae were motionless after transfer from 13 °C to the warm incubator, but live ones began to move within a few hours. After 24 h in the incubator, the number of surviving larvae in each plate was determined by washing the media through a sieve and placing the retained larvae on a perforated stainless steel plate with holes sufficiently large for them to crawl through. Dead

larvae remained on the surface of the plate, whereas live ones quickly penetrated the holes and fell into a catch tray under it. The number of surviving larvae was recorded for each treatment at each time period.

Second instar larvae were utilized to determine the number of days to a 100% kill in LP. Five replicate petri dishes, each containing about 300 larvae, were placed in a 25 °C, high-humidity incubator to determine survival rates of nontreated larvae. Groups of five dishes were each loosely wrapped in reflective Mylar film. Thirty-five dishes were placed in each 15 mm Hg LP, and AP desiccator maintained at 13 °C. After 2, 4, 6, 8, 10, 12, and 14 d, five larvae-containing petri dishes were transferred from the LP and AP desiccators into pupation bowls for incubation in the 25 °C incubator. Surviving larvae were counted as described above.

STORAGE OF TROPICAL FRUIT. Small-scale trials were conducted on tropical fruits grown in the local area to determine the efficacy of LP storage for various periods of time. Fully mature, near ripe 'Keitt' mangoes and 'Arkin' carambolas (*Averrhoa carambola*) were placed in the LP (15 mm Hg at 13 °C) and AP control desiccators (13 °C), each for 2 months, and 'Thai' guava (*Psidium guajava*) was stored for 1 month. In each trial, the containers were opened periodically to determine if any fruit were ripening. Sample fruit were removed for taste tests and the containers resealed.

DATA ANALYSIS. The number of eggs and larvae surviving each sampling time in LP or AP in each replicate petri dish were first ranked from lowest to highest surviving larvae numbers in the pupation bowls within each experimental run to account for the range in original numbers of eggs inoculated into each dish. The ranked number of survivors per dish for time elapsed in each condition was then normalized to a proportion (percentage) of the similarly ranked numbers per dish of surviving nontreated eggs or larvae using Abbott's formula (Abbott, 1925). In rare cases where the 4-d number of surviving eggs in AP was greater than the correspondingly ranked original, the latter was adjusted to equal the former. The normalized data were averaged and standard error calculated among replicate dishes for each treatment-time period and across

the three experimental runs of the 20 mm Hg LP egg data, six runs of the 15 mm Hg egg data, and within the two 15 mm Hg larvae experiments. The egg mortality results in AP were combined across all nine runs of the LP experiments and averaged, since the conditions for AP were the same in both experiments.

A probit analysis [PROC GENMOD (SAS for Windows version 9.1; SAS Institute, Cary, N.C.)] was used to analyze the mortality rates of fruit fly eggs over time in response to the 15 and 20 mm Hg LP treatments to predict the time required to achieve 99.999% egg mortality in each condition. There are three parts to the statistical analysis of the data from these experiments. The first part involved determining the appropriate scale (linear or natural log transformed) for time to be used in the mortality model. The second part was to test whether the data from the 15 or 20 mm Hg LP treatment group had similar time patterns to the 760 mm Hg AP group. The third part was to develop a model useful for predicting percent mortality as a function of time for each treatment group.

To determine the appropriate scale, four models were examined. Two models involving time as a covariate, Model A (treatment, time, treatment $\times$ time interaction) and Model B (treatment, log-time, treatment $\times$ log-time interaction), were fit to the data and overall goodness-of-fit (GOF) of the two models compared. The treatment factor in the models was coded 1 for the 760 mm Hg AP group and 0 for the LP treatment group. Similarly, two other models were fit to the treatment data only, Model C (time alone) and Model D (time on a natural log scale), and GOF between these two models compared. A deviance statistic adjusted for appropriate degrees of freedom was used to assess overall GOF and aid in comparing Model A to Model B and for comparing Model C to Model D. If Model B and Model D had smallest deviance, then time would be transformed by the natural log for subsequent analyses; otherwise untransformed time would be used.

In the first step, it was determined that Model B had smaller deviance than Model A, and Model D smaller deviance than Model C; hence a natural log transformation of exposure times (the predictor) was used in the subsequent analysis. In the second part, it

was determined that Model B fit better than Model A, and hence Model B was used to test for treatment effects. Both Model C and Model D were developed for prediction purposes. Whereas the overall fit of Model D was slightly better than Model C, residual analysis indicated that Model C would be the better model for prediction purposes. Therefore, the Model C for 15 mm Hg LP treatment data was used in the generation of the Probit 9 egg mortality prediction for that treatment, and a separate Model C developed for the 20 mm Hg LP treatment data used for Probit 9 prediction for that treatment.

Results

We found it difficult to reduce variability in the number of eggs inoculated on each petri dish, both within an experimental run and from run to run. Normalization of the data at each time interval to a corresponding proportion of the nontreated eggs in the LP and AP treatments allowed comparison among replicate plates within the same experimental run and among experimental runs.

EGGS EXPOSED TO LP AND AP. The first experiments were conducted comparing mortality of eggs stored at 13 °C under 20 mm Hg LP vs. AP. Figure 1 presents the average proportion of eggs killed after various time intervals in LP and AP. An average of about 75% of the eggs were killed in the first 4 d of LP treatment compared to only 20% over the same period of exposure in AP. All of the eggs were killed by 11 d exposure to 20 mm Hg. Mortality of eggs stored at 15 mm Hg increased slightly faster than those exposed to 20 mm Hg (Fig. 1). Despite the initially high mortality rate of 80% by the fourth day in LP, a small population of resilient eggs withstood the vacuum and temperature conditions for up to 9 d. Eggs kept in AP died at a linear rate with only 75% being killed after 14 d. There was little variance in the mortality results at each time period, as indicated by the low standard errors in both the LP and AP treatments.

LARVAE EXPOSED TO 15 MM HG. The initial larvae experiment indicated that first and second instar larvae were equally susceptible to the 15 mm Hg LP treatment (data not shown), so the second experiment investigating the time necessary for 100% mortality was conducted using second instar

larvae. Larvae appear to be more sensitive than eggs to 15 mm Hg, as evidenced by greater mortality at 4 d; however, mortality in LP could also be influenced by the greater apparent sensitivity of larvae to cool temperatures, as evidenced by the AP results (Fig. 2). All larvae were killed by the 10th day of exposure to both the LP and AP conditions at 13 °C.

ASSESSMENT OF TIME SCALE AND TREATMENT EFFECTS. In general, Model A [GOF = 19.9 for 15 mm Hg and 32.9 for 20 mm Hg (GOF = deviance/df)] displayed much poorer overall fit than Model B (GOF = 16.6 for 15 mm Hg and 30.8 for 20 mm Hg), and Model C (GOF = 7.3 for 15 mm Hg and 13.2 for 20 mm Hg) only slightly poorer than Model D (GOF = 4.2 for 15 mm Hg and 9.2 for 20 mm Hg). It was concluded that a natural log scaled time measure would be used for the assessment of treatment effects.

The data from the 15 mm Hg LP and 760 AP treatments were compared using Model B (treatment, natural log time, and treatment $\times$ log time interaction) and a separate Model B used to compare the 20 mm Hg LP treatment to the AP treatment. The parameter estimates for Model B for both groups are provided in Table 1. As expected, there was a significant time trend ($P < 0.001$) for both the 15 and 20 mm Hg LP models. Significant interaction effects with negative parameter estimates in both the 15 and 20 mm Hg treatments demonstrated that the treated eggs had significantly lower expected survival rates than those of the AP group ($P < 0.001$ for both experiments).

FIT TO LP-TREATED EGG DATA.

Model C was used to estimate a survival-predicting curve for treated eggs with separate models fit to the 15 and 20 mm Hg levels of LP (Table 2). For the 15 mm Hg treatment, at 9 d exposure, 99.99645% of the eggs were predicted to be dead with 95% confidence interval of 99.99512% to 99.99743%. For the 20 mm Hg treatment, the model predicted 99.99967% mortality of caribbean fruit fly eggs at 11 d exposure with 95% confidence interval of 99.99942% to 99.99982%. The Probit 9 analysis to estimate time to 99.999% probable kill (95% confidence range) indicated it would take 9.44 ± 0.12 d exposure to 15 mm Hg and 10.60 ± 0.20 d exposure to 20 mm Hg to obtain this level of egg mortality.

Fig. 1. Average ($\pm$ SE) mortality of caribbean fruit fly eggs exposed to 15 mm Hg (2.0 kPa), 20 mm Hg (2.7 kPa) low pressure (LP) and 760 mm Hg (101.3 kPa) standard atmospheric pressure (AP) at 13 °C (55.4 °F) over time. Data expressed as proportion of the original number of eggs. Some SE bars are hidden by data marks.

Fig. 2. Average ($\pm$ SE) mortality of caribbean fruit fly larvae exposed to 15 mm Hg (2.0 kPa) low pressure (LP) and 760 mm Hg (101.3 kPa) standard atmospheric pressure control (AP) at 13 °C (55.4 °F) over time. Data expressed as proportion of the original number of larvae. Some SE bars are hidden by data marks. Visible SE bar at 2 d refers to 760 mm Hg datum.

FRUIT STORAGE. The fully mature 'Keitt' mangoes from a local orchard that were placed in the four LP desiccators and two AP desiccators ripened normally. All of the AP-treated fruit ripened within 10 d. The flavor of fruit that ripened at room temperature after up to 50 d in LP rivaled that developed by freshly harvested fruit.

Carambolas from a commercial orchard were transferred directly to the LP and AP desiccators without surface sterilization with chlorine,

as is customary prior to commercial shipment. Fruit stored in the AP desiccators were overwhelmed with fungal rot within several days. Those stored in the LP desiccators were generally in the same condition after 2 months storage as they were when placed in the containers except for small spots of fungal mycelia on the surface of about 40% of the fruit. Guavas stored in LP were unchanged after 1 month, whereas those stored in the AP desiccators softened in about 2 weeks.

Table 1. Estimated parameter values for Model B [involving time as a covariate (i.e., treatment, log-time, treatment $\times$ log-time interaction) with associated SE and *P* values for Type III (directional error) tests for the hypothesis that parameter values are equal to zero for the influence of 15 and 20 mm mercury (Hg)^z absolute pressure on the mortality of caribbean fruit fly eggs from all experiments involving 15 or 20 mm Hg treatments combined (respectively) at 13 °C (55.4 °F).

Parameter	Estimate	SE	<i>P</i>
<i>15 mm Hg treatment</i>			
Intercept	-2.5959	0.0716	<0.0001
Treatment (AP) ^y	0.8294	0.6768	<0.0001
Log-time	2.4144	0.0380	<0.0001
Log-time $\times$ treatment (AP)	-1.5437	0.0489	<0.0001
<i>20 mm Hg treatment</i>			
Intercept	-2.5585	0.0876	<0.0001
Treatment (AP)	0.0271	0.0970	0.7798
Log-time	2.2282	0.0557	<0.0001
Log-time $\times$ treatment (AP)	-0.9792	0.0590	<0.0001

^z1 mm Hg = 0.1333 kPa.

^yTreatment (AP) implies that the treatment factor in the model is coded as 1 for the 760 mm Hg AP group and 0 for the LP treatment group.

Discussion

Increased storage life of fresh fruits and vegetables in LP is well established (Alvarez, 1980; Burg, 1990, 2004). Preservation is pressure dependent. For example, mango storage life was slightly prolonged at 150 to 200 mm Hg (Burg and Burg, 1966). It was extended to 4 weeks at 75 to 80 mm Hg (Apelbaum et al., 1977b; Spalding, 1977; Spalding and Reeder, 1976), to more than 5 weeks at 50 mm Hg (Apelbaum et al., 1977b), and more than 6 weeks at 25 mm Hg (D. Spalding, personal communication). The optimal LP storage pressure (15 to 25 mm Hg) and temperature (10 to 13.3 °C) is similar for mangoes, papayas (*Carica papaya*) (Jamieson, 1980a, 1980b), avocados (*Persea americana*) (Burg, 2004; Cicali and Jamieson, 1978; Dressler, 1978), and bananas (*Musa $\times$ paridasiaca*) (Apelbaum et al., 1977a; Burg, 2004). The small-scale trials conducted on the three locally available fresh tropical fruits, mango, carambola, and guava confirmed these observations and verified that the LP conditions created in the present experiments were suitable for fruit storage as well as killing fruit fly eggs and larvae.

The caribbean fruit fly mortality results presented here are qualitatively similar to those obtained when other insect species have been subjected to vacuum treatment. A sample of 120 papayas that had been inoculated with papaya fruit flies was divided equally between an LP container operated at 20 mm Hg, 10 °C, and a standard refrig-

erated normal atmosphere container controlled at the same temperature. No flies hatched when the fruits were removed and ripened after 3 weeks of LP storage, whereas numerous flies hatched after conventional cold storage (A.M. Alvarez, personal communication). Adult and larval forms of fruit flies and other insects, but not eggs or pupae, were killed by a 48-h exposure to a pressure of about 50 mm Hg (A. Apelbaum, personal communication).

Navarro and Calderon (1979) determined that low oxygen tension under vacuum, rather than desiccation or low pressure per se, is the cause of insect mortality. Low oxygen tensions that are lethal to insects are injurious to plant commodities at atmospheric pressures, but not in vacuum (Burg, 1990, 2004; Hatton and Spalding, 1990). Mangoes, for example, store optimally in <0.3% O₂ in LP conditions whereas damage occurs to the fruit if the O₂ tension is below 5% at atmospheric pressure (Burg, 1990). In our experiments, the saturation of water in the rarified atmosphere of the LP containers and normal atmosphere of the AP containers prevented any evaporation of water from the media containing the eggs or larvae. Careful attention to prevention of vacuum leaks and shielding the media from incident radiation using the reflective Mylar film were the keys to retaining water in the media.

Our results from three replicate runs with eggs treated at 20 mm Hg LP and from six replicate runs at 15

Table 2. Parameter estimates for the Model C (based on time only) mortality-prediction curve of caribbean fruit fly eggs over time exposed to 15 and 20 mm mercury (Hg)^z low pressure (LP) treatments at 13 °C (55.4 °F).

Parameter	Estimate	SE	<i>P</i>
<i>15 mm Hg</i>			
Intercept	-2.1319	0.0285	<0.0001
Time	0.6783	0.0070	<0.0001
<i>20 mm Hg</i>			
Intercept	-2.0351	0.0365	<0.0001
Time	0.5947	0.0085	<0.0001

^z1 mm Hg = 0.1333 kPa.

mm Hg LP (Fig. 1) were highly significant in that Probit 9 analysis of the data predicted virtually complete kill by 9.44 and 10.60 d exposure to 15 and 20 mm Hg LP, respectively. Although the larval mortality results in 15 mm Hg (Fig. 2) were highly consistent in both time to kill and low variance with those of the eggs (Fig. 1), insufficient replicate runs were conducted on the larvae to allow a meaningful Probit 9 analysis. The impact of cool temperature alone on mortality of caribbean fruit fly larvae in AP overshadows the potential usefulness of LP for killing caribbean fruit fly larvae that are imbedded in tropical fruit. It is, however, the temperature-resilient eggs present in fruit at harvest that must be controlled, not larvae. LP provides the technology to accomplish this. Although quantitative research on mortality of fruit fly eggs in fruits has yet to be done, this is the first report to demonstrate the potential to kill tropical fruit fly eggs and larvae in such fruit while in transit, and it is the first clear demonstration of Probit 9 prediction of insect disinfestation within the same time frame as typical overseas shipments. The LP treatment is, thus, especially useful as a quarantine treatment since it can be achieved during shipment of fruit to destination markets while assuring optimum fresh fruit quality. It has the potential to eliminate the need for costly and occasionally damaging hot-water treatments specifically accepted for certain mangoes (Sharp, 1986; Sharp and Hallman, 1994; Sharp and Spalding, 1984; Sharp et al., 1988, 1989a, 1989b) and to provide in-transit treatments providing quarantine insect control at levels required by USDA/APHIS for all species of fruit flies in tropical fruits of all kinds and sizes.

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