Mortality of Caribbean Fruit Fly (Diptera: Tephritidae) Immatures in Coated Fruits

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ABSTRACT The fruit coatings Primafresh 31, Sta-Fresh 360HS, Sta-Fresh 600, and Nature Seal were studied for their ability to kill Caribbean fruit fly, *Anastrepha suspensa* (Loew), larvae infesting fruits. Mortality in grapefruits ranged from 33% (Primafresh 31) to 100% (Sta-Fresh 600). Methyl cellulose and shellac, known to restrict gaseous exchange, were identified as substances in Nature Seal that reduced survival of Caribbean fruit fly; Nature Seal with 4% methyl cellulose and 17% shellac caused 99.2% mortality in grapefruits. Coating of cold-stored mangoes and carambolas did not increase Caribbean fruit fly mortality. Hot-air treatment of Nature Seal (2% methyl cellulose plus 10% shellac)-coated grapefruits increased Caribbean fruit fly mortality compared with uncoated fruits; when both were exposed to hot air at 48°C for 60 min, no larvae survived treatment of coated grapefruits, whereas 24% survived treatment of uncoated grapefruits. Carambolas immersed in 46°C Nature Seal heated slower than those immersed in 46°C water. Also, more damage occurred to carambolas immersed in Nature Seal than in water at 46°C. Carbon dioxide and alcohol levels were higher in coated grapefruits, and oxygen levels were lower compared with uncoated grapefruits. Coatings may have potential as possible quarantine treatments of interior pests, if not alone then in combination with other treatments. Coatings might also be considered as part of an integrated pest management system of fruit pests.

KEY WORDS controlled atmosphere, modified atmosphere, fruit wax

MANY FRUITS ARE waxed or coated to prolong shelf life. Coatings prolong shelf life by restricting the exchange of oxygen, carbon dioxide, and water vapor; this reduces the rates of metabolism and water loss in the fruit (Kester & Fennema 1986). Coatings are thus similar to controlled atmosphere storage in which commodities are kept in atmospheres low in oxygen and high in carbon dioxide to lower fruit metabolism. Controlled atmosphere quarantine treatments have been developed such that the atmospheric conditions in the storage facility are toxic to pests (Hallman 1994). By restricting gaseous exchange between the fruit and its surroundings and, thus, altering gas concentrations inside the fruit, use of coatings might result in mortality to insects inside the fruit.

Coating of oranges has been shown to increase and reduce the internal levels of carbon dioxide and oxygen, respectively (Nisperos-Carriedo et al. 1990). In studies on the effects of methoprene-impregnated waxes on Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), immatures inside papayas and peaches, Saul et al. (1985, 1987) presented data which indicated that the waxes by themselves might provide significant mortality. Controls composed of fruits coated with wax not containing methoprene were included; estimated mortalities of Mediterranean fruit flies were 20–89%.

Coatings have been approved as a quarantine treatment for cherimoya, *Annona cherimola* Mill., fruit from all countries and limes from Chile shipped to the United States and infested with the mite *Brevipalpus chilensis* Baker (Anonymous 1993). However, the mode of action of a coating against mites or other pests on fruit surfaces probably involves physical constraint of the mites and sealing of the tracheal and alimentary systems rather than restriction of gaseous exchange as in a controlled atmosphere treatment.

The objective of the research reported here was to test coatings for their ability to kill Caribbean fruit fly, *Anastrepha suspensa* (Loew), immatures inside various types of fruit when used alone or in combination with other treatments.

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

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Materials and Methods

Grapefruits were obtained from Seald Sweet Growers, Vero Beach, FL, and DNE World Fruit Sales, Fort Pierce, FL; carambolas and mangoes were obtained from J. R. Brooks & Son, Homestead, FL. The fruits were infested with immature Caribbean fruit flies by placement in an outdoor screen cage (3.5 by 3.5 by 2.5 m) with ≈200,000 flies. The flies were reared on an agar-based diet (85% of the flies) and a corn cob-based diet (15%) (Burditt et al. 1975). Temperature within the cage ranged between 21 and 32°C. After removal from the infestation cage, fruits were washed and coated.

We used four coatings. Nature Seal is an edible composite coating containing methyl cellulose and emulsifiers that was developed at the U.S. Citrus and Subtropical Products Laboratory in Winter Haven, FL (Nisperos-Carriedo & Baldwin 1993). Sta-Fresh 360HS (FMC, Riverside, CA) contains alkali-soluble resins, denatured alcohol, food-grade surfactants, and silicone antifoam. Sta-Fresh 600 (FMC) is a nondrying coating composed of food-grade emulsifiers and surfactants. Primafresh 31 (S. C. Johnson & Son, Racine, WI) contains amine-fatty acid soap, waxes, and food-grade shellac. Nature Seal and Primafresh 31 are virtually invisible once they dry on the fruit, whereas Sta-Fresh 360HS imparts a glossy finish. Coatings were applied by submerging each fruit in a bowl of the coating at 23 ± 0.5°C, then placing the fruits on a rack to dry.

Fruit Fly Mortality. Several different experiments were done with the coatings. In the first experiment, grapefruits were infested for 6 d, then cleaned with ambient tap water and scrubbing rollers. One day later they were immersed in Sta-Fresh 600, Sta-Fresh 360HS, Primafresh 31, or Nature Seal (2% methyl cellulose) for 3 s, removed and allowed to dry on racks. The grapefruits were placed in metal towers above bins containing sand; surviving third instars dropped into the sand and pupated. The sand was sifted weekly. Grapefruits with no coating were also included in the test; the number of larvae emerging from these fruits was used as to estimate the number of larvae present in the coated grapefruits. Six replicates of 40 grapefruits were used per treatment. Data were analyzed as the number of larvae emerged per treatment with the SAS GLM procedure (SAS Institute 1989); results are presented as percentage mortality.

Another experiment was designed to test the effect of different amounts of methyl cellulose and shellac in Nature Seal on survival of Caribbean fruit fly immatures. Grapefruits were infested and coated with Nature Seal containing 1, 2, or 4% methyl cellulose or 4% methyl cellulose plus 17% food-grade shellac. A control with no coating was also used; four replicates of 40 grapefruits were used per treatment. The number of larvae emerged per treatment (n) was transformed by $\log(n + 1)$ before analysis with the SAS GLM procedure (SAS Institute 1989). Results are presented as percentage mortality.

Mangoes cannot be stored at temperatures sufficiently low to provide quarantine security from fruit flies (<5°C) without damaging fruit quality. An experiment was conducted to determine if normal storage temperatures, combined with a coating, would increase the mortality of Caribbean fruit fly immatures inside mangoes. Mangoes ('Tommy Atkins', mean weight, 296 g; range, 157–444 g) were placed in the screened infestation cage at 24–34°C for 3 d, removed, washed, and stored for 3 d at ≈24°C. The fruits were then randomly divided into nine equal groups, four each of which were dipped in Nature Seal containing 0.75 or 1.0% methyl cellulose. The uncoated group of mangoes and one group each of the coated mangoes were immediately placed in cages at ≈24°C to collect surviving larvae. The next day, the other six groups of mangoes were placed in a chamber at 13 ± 0.5°C. One group of mangoes per each coating was removed from the chamber at 4, 8, or 11 d and placed in cages to collect survivors. The number of larvae emerging from the uncoated group of mangoes was used to approximate the number of Caribbean fruit fly immatures present in each group of treated mangoes to estimate mortality. Three replicates of 37, 42, and 44 mangoes per treatment were done. Regression analysis (SAS Institute 1989) was used to determine if mortality of Caribbean fruit fly in mangoes increased with increasing number of days at 13°C. The regression equation was mortality equals days at 13°C.

Carambolas are currently treated with 1°C storage for 15 d as a quarantine treatment against Caribbean fruit fly (Gould & Sharp 1990). To determine if the duration of the cold treatment could be shortened, carambolas ('Arkin'; mean weight, 138 g; range, 70–182 g) were put in the infestation cage at 18–28°C for 3 d, wiped clean with cotton cloth, and stored for 3 d at ≈24°C. Half of the fruits were coated with Nature Seal (2% methyl cellulose). After 1 d, the coated and uncoated carambolas were each randomly divided into six groups of 40 carambolas each and placed in a chamber at 1 ± 0.3°C for 2, 4, 6, 8, or 11 d. The sixth group was placed in cages to determine population levels in the fruits without the cold treatment. After removal from the cold chamber, each group of carambolas was caged to determine Caribbean fruit fly survival. The experiment was repeated three times; data were subjected to probit analysis (SAS Institute 1989).

Mangoes are immersed in water at 46.1°C for 75–90 min to kill fruit fly immatures (Anonymous 1990). An experiment was done to determine if fruits could be immersed in hot coating as a quarantine treatment. The coating might give higher

mortality levels than water, or help preserve fruit quality after the immersion; hot-water immersion shortened the shelf life of carambolas (Hallman 1989). Carambolas were infested as described before. When third instars were present, the carambolas were randomly separated into 11 groups of 40 fruits each. One group was caged and larvae allowed to emerge. These larvae were counted and used to estimate the number of larvae present in the other 10 groups. One group each was immersed in water or Nature Seal (1.5% methyl cellulose) at 46°C for 5, 10, 15, 20, or 25 min before being placed in cages to recover surviving larvae. The experiment was done twice, and data were subjected to probit analysis.

Forced hot air (48°C) has been developed as a quarantine treatment for grapefruits infested with Caribbean fruit fly (Sharp 1993). However, McGuire & Reeder (1992) concluded that late-season grapefruits would not tolerate forced hot air treatments >47.5°C for the length of time necessary to ensure mortality of all immatures. Therefore, research was conducted to determine if forced hot air treatment of coated grapefruits could be done more quickly than treatment of uncoated grapefruits. Heat often interacts synergistically with controlled-atmosphere quarantine treatment (Hallman 1994). Grapefruits were placed in an infestation cage at 18–28°C for 6 d, cleaned, and then held at ≈24°C for 2 d before being randomly divided into nine groups of 42 fruits each. Four of the groups were coated with Nature Seal containing 2% methyl cellulose and 10% food-grade shellac. The next day the four coated groups plus four of the uncoated groups were subjected to forced hot air at 48°C for 60, 90, 120, or 150 min with the machinery described by Sharp et al. (1991). The ninth group was placed in a cage to recover larvae, which were used to estimate the number of larvae present in the treated groups. The experiment was replicated three times.

Gases and Volatiles in Coated Grapefruits. Fifty-five grapefruits were exposed to Caribbean fruit fly oviposition for 2 d. After one more day, five infested grapefruits plus five uninfested grapefruits were coated with Nature Seal (4% methyl cellulose, 17% shellac) or Sta-Fresh 360HS. Five infested and uninfested grapefruits were not coated. The fruits were stored at 21°C for 7 d and then sampled for internal oxygen, carbon dioxide, acetaldehyde, methanol, and ethanol as described by Nisperos-Carriedo et al. (1990). The remaining 40 infested grapefruits were placed in a cage to recover larvae as an estimate of the infestation rate in the grapefruits examined for gases and volatiles. Data were analyzed with the SAS GLM procedure. Means were compared with the Ryan-Einot-Gabriel-Welch multiple F test (SAS Institute 1989).

Table 1. Percentage mortality of Caribbean fruit fly larvae emerging from uncoated grapefruits and grapefruits coated with four coats after infestation

Coat	% Mortality
Sta-Fresh 600	100a
Sta-Fresh 360HS	88ab
Nature Seal (2% methyl cellulose)	72b
Primafresh 31	33c
No coat	0d

Values followed by same letter are not significantly different at the 95% confidence level (Ryan-Einot-Gabriel-Welsh multiple range test [SAS Institute 1989]). No coating was assumed to be 0% mortality; the number of larvae emerging from it (1,128) was used to estimate the number of larvae per treatment.

Results and Discussion

Fruit Fly Mortality. Significantly fewer Caribbean fruit fly larvae emerged from coated than from uncoated grapefruits; differences among coatings were significant (Table 1). No larvae of an estimated 1,128 survived in grapefruits coated with Sta-Fresh 600. Hagenmaier & Shaw (1992) found that fruit coatings varied considerably in their permeability to oxygen and carbon dioxide. Survival of Caribbean fruit flies in grapefruits coated with Nature Seal was related inversely to cellulose content (Table 2). The addition of 17% shellac reduced survival to 0.8% of an estimated 3,021 larvae.

Mean survival of Caribbean fruit fly larvae in mangoes coated with Nature Seal containing 1 and 0.75% methyl cellulose was 7.5 and 48.5%, respectively. Mortality did not increase with increasing days of storage at 13°C; regression analysis showed that the slope was not significantly different from zero in both coatings. Values of F and correlation coefficients for the 0.75% methyl cellulose Nature Seal coating were 0.8835 and 0.0025, respectively. For the 1.00% methyl cellulose coating, the F probability and correlation coefficient were 0.809 and 0.0061, respectively.

A Nature Seal coating containing 2% methyl cellulose did not provide significantly higher mortality of Caribbean fruit fly larvae in caram-

Table 2. Percentage mortality of Caribbean fruit fly larvae that emerged from grapefruits coated with formulations of Nature Seal after infestation

Nature Seal formulation	% Mortality
4% cellulose + 17% shellac	99.2a
4% cellulose	80.7b
2% cellulose	70.8bc
1% cellulose	26.2cd
No coat	0.0d

Values followed by the same letter are not significantly different at the 95% confidence level (Ryan-Einot-Gabriel-Welsh multiple range test [SAS Institute 1989]). No coat was assumed to be 0% mortality; the number of larvae emerging from it (3,021) was used to estimate the number of larvae per treatment. Data (number of larvae emerging per treatment) were transformed by log (n + 1) before analysis.

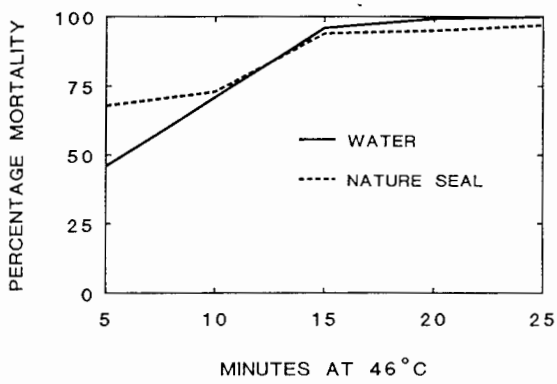

Fig. 1. Mortality of Caribbean fruit fly larvae in carambolas immersed in water or Nature Seal (1.5% cellulose) at 46°C for up to 25 min.

bolas stored at 1°C compared with mortality in uncoated carambolas. Results of probit analysis for Caribbean fruit fly mortality in uncoated carambolas were as follows: X^2 probability <0.001; slope ± SEM = 0.42 ± 0.097; $LT_{99.9968}$ = 12.1 d (95% FL = 9.2–21.8 d). The slope and $LT_{99.9968}$ were similar to those reported by Gould & Sharp (1990) for carambolas stored at 1.1°C. Results of probit analysis for Caribbean fruit fly larval mortality in carambolas coated with Nature Seal (2% methyl cellulose) were as follows: X^2 probability <0.001; slope ± SEM = 0.39 ± 0.058; $LT_{99.9968}$ = 10.8 d (95% FL = 8.9–14.8 d). After 2 d at 1°C, mortality of Caribbean fruit flies in coated and uncoated grapefruits was 71.8 and 55.2%, respectively. After 8 d at 1°C, however, mortality of Caribbean fruit flies was identical (99.68%) in coated and uncoated carambolas.

Mortality of immature Caribbean fruit flies was greater in infested carambolas immersed in Nature Seal at 46°C than in water at the same temperature for immersion times of 5 and 10 min (Fig. 1). After an immersion time of 10 min, mortality was greater in the carambolas immersed in water. The carambolas heated more slowly in Nature Seal than in water. Compared with hot water up to 10 min, the higher mortality of Caribbean fruit flies in carambolas immersed in hot coating was probably the result of the modified atmosphere property of the coating, and not heat. After 10 min, the higher temperatures in the fruits subjected to hot water immersion versus hot coating immersion resulted in higher mortality in the hot water immersed carambolas.

No larvae (of an estimated 384 per treatment time) emerged from grapefruits that were coated with Nature Seal (2% methyl cellulose, 10% shellac) and treated with hot air at 48°C for ≥60 min. Mortality of Caribbean fruit flies in uncoated grapefruits exposed to hot air (three replicates) for 60 min was estimated to be 59, 84, and 85% and 96, 97, and 100% in uncoated grapefruits heated for 90 min. No Caribbean fruit flies emerged from uncoated grapefruits heated for 120 and 150 min.

Table 3. Mean gas and volatile concentrations in 'Marsh' white grapefruits uncoated or coated with Nature Seal (4% methyl cellulose plus 17% shellac) or Sta-Fresh 360HS

Treatment	Concentrations[a]			
	Co_2, %	O_2, %	Methanol, ppm	Ethanol, ppm
No coat	1.7a	11.6a	169a	455a
Nature Seal	9.6b	4.3b	420ab	2,517b
Sta-Fresh 360HS	13.1c	4.2c	502b	4,023b

Values in each column followed by same letter are not significantly different at the 95% confidence level (Ryan-Einot-Gabriel-Welsh multiple range test [SAS Institute 1989]).

Gases and Volatiles in Coated Grapefruits. Coated grapefruits had significantly higher levels of carbon dioxide and lower levels of oxygen than uncoated grapefruits (Table 3). Mean concentrations of carbon dioxide were 1.7, 9.6, and 13.1% for uncoated, Nature Seal-coated, and Sta-Fresh 360HS-coated grapefruits, respectively; all three means were different from each other at the 95% confidence level (Ryan-Einot-Gabriel-Welsch multiple F test). Mean oxygen concentrations were 11.7, 4.3, and 4.2% for uncoated, Nature Seal-coated, and Sta-Fresh 360HS-coated grapefruits, respectively; the mean for percentage oxygen in the uncoated grapefruits was significantly different (95% CL) from the means for the coated fruits.

Benschoter (1987) achieved 43.3–100% mortality of Caribbean fruit fly eggs and larvae in agar diet exposed to oxygen levels of 2 or 10% and carbon dioxide levels of 20% at 10 or 15.6°C. Levels of methanol and ethanol were also significantly higher in coated fruits compared with uncoated fruits (Table 3), a factor that also might contribute to fruit fly mortality. Nisperos-Carriedo et al. (1990) found a moderate increase in acetaldehyde in coated pineapple oranges; acetaldehyde (1–1.5%) caused significant mortality of green peach aphid, *Myzus persicae* (Sulzer), in lettuce, and its effect was synergized by controlled atmospheres (Hartsell et al. 1979). Acetaldehyde levels were not significantly affected in our study (Table 4). A mean of 4.4 Caribbean fruit fly larvae per grapefruit emerged from the 40 uncoated grapefruits. We detected no significant differences between infested and uninfested fruits for any of the compounds measured, nor were the interactions between coating and fly infestation significant for carbon dioxide and oxygen levels (Table 4).

In conclusion, coatings caused significant mortality to Caribbean fruit fly larvae infesting fruits. We identified methyl cellulose and shellac, which are known to restrict gaseous exchange, as substances that were detrimental to survival of Caribbean fruit flies in coated fruits.

Table 4. Internal gas and volatile concentrations in 'Marsh' white grapefruits infested or not infested with Caribbean fruit fly (mean of 4.4 larvae per fruit) and uncoated or coated with Nature Seal (4% methyl cellulose plus 17% Shellac) or Sta-Fresh 360HS

Factor analyzed	F	df	$P > F$
Carbon dioxide by coat × fly infestation	1.7	2, 5	0.27
Carbon dioxide by coat	51.9	2, 8	0.0001
Carbon dioxide by infestation	2.9	1, 8	0.17
Oxygen by coat × infestation	0.81	1, 4	0.42
Oxygen by coat	12.9	2, 7	0.0045
Oxygen by infestation	3.3	1, 7	0.14
Acetaldehyde by coat	1.8	2, 6	0.24
Acetaldehyde by infestation	0.88	1, 3	0.42
Methanol by coat	22.2	2, 5	0.0032
Methanol by infestation	1.9	1, 3	0.26
Ethanol by coat	8.9	2, 5	0.022
Ethanol by infestation	2.1	1, 2	0.28

Coating of cold-stored fruits did not increase Caribbean fruit fly mortality. Usually, controlled atmosphere does not shorten the duration of a cold treatment (Hallman 1994). Insects require less oxygen at low temperatures than at high temperatures; therefore, a reduction in oxygen uptake by a coated fruit at cold temperatures may not be as detrimental to insects infesting the fruit as it would be at higher temperatures. Coating of grapefruits treated with hot air increased mortality of Caribbean fruit flies compared with uncoated fruits; synergism of heat and controlled atmosphere for insect control is known to occur, perhaps as a result of increased oxygen consumption and carbon dioxide production of insects at higher temperatures (Hallman 1994). Carambolas immersed in 46°C Nature Seal heated more slowly than those immersed in 46°C water, resulting in lower levels of mortality with the Nature Seal immersion. Preliminary studies showed that carambolas immersed in 46°C Nature Seal for 55 min (which is estimated to fall short of providing $LT_{99.9968}$ quarantine security) showed considerable damage which resulted in premature decomposition.

Coatings have potential for use as quarantine treatments, if not by themselves then in combination with other treatments or as part of a systems approach to quarantine security (Jang & Moffitt 1994). Coatings should also be tried as a post-harvest component of an integrated pest management system of fruit pests without regard to quarantine purposes.

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