Vapor Heat Treatment for Grapefruit Infested with Caribbean Fruit Fly (Diptera: Tephritidae)

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ABSTRACT 'Marsh' grapefruit, *Citrus paradisi* Macf. (size #36, mean weight = 426.9 ± 80.0 g) were subjected to vapor heat (saturated hot air) to determine the effect of the treatment on grapefruit quality and the mortality of larvae of the Caribbean fruit fly, *Anastrepha suspensa* (Loew), inside the grapefruit. Vapor heat at 46–46.4°C for 3.75 h resulted in drying of the oil glands of the grapefruit peel; rot of uninfested fruit increased to 9.4% after 60 d storage compared with 2.9% for untreated, uninfested fruit. Vapor heat at 43.3–43.7°C for 5 h caused no damage to fresh grapefruit. Probit 9 (99.9968%) mortality of larvae of *A. suspensa* in grapefruit treated with vapor heat at 43.3–43.7°C was estimated to be 252.0 min (95% FL = 235.3–272.4 min). When approximately 103,470 larvae (mostly third instars) of *A. suspensa* were subjected to vapor heat at 43.3–43.7°C for 270 min, three larvae survived; two of these died later as pupae. We recommend that #36 grapefruit infested with *A. suspensa* be subjected to vapor heat at 43.3–43.7°C until the centers of treated fruits remain at 43.3°C for 50 min.

KEY WORDS Insecta, *Anastrepha suspensa*, commodity treatment, quarantine security

THE VAPOR HEAT quarantine treatment uses heated air saturated with water vapor to heat fruits to a specified temperature for a required time to kill virtually all tephritid fruit fly immatures in the fruits (USDA-APHIS 1985). Current guidelines for grapefruit, *Citrus paradisi* Macf., infested by the Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae), require that the temperature at the center of the grapefruit be raised to 43.3°C over a period of 8 h and maintained at that temperature for 6 additional hours (USDA-APHIS 1985).

The objective of our research was to determine if vapor heat treatments <14 h would provide probit 9 security (99.9968% mortality) against larvae of Caribbean fruit fly, *Anastrepha suspensa* (Loew), in grapefruit.

Materials and Methods

Vapor Heat Apparatus. A vapor heat treatment facility was constructed at the USDA Subtropical Horticulture Research Station, Miami. An electric steam humidifier (Vapormist Model VM10, DRI-STEEM, Hopkins, Minn.) was used to generate steam into an insulated Modular Thermopanel chamber (2.4 by 2.8 by 2.5 m; Inter-American Commercial Refrigeration, Miami). The chamber stored air at the proper conditions of 100% RH and 43.3–43.6°C temperature. An electric fan (G-1282, Patton Electric Company, New Haven, Ind.) set on high provided turbulence inside the chamber and mixed the incoming air and steam.

An aluminum case (2 m long by 0.5 m^2, insulated by 2 cm of Styrofoam) with a hinged top was used to hold the fruit. One end of the aluminum case was exposed to the inside of the large chamber. It was closed during warm-up by a urethane foam plug (0.4 m^2 by 0.1 m thick) to prevent the fruit from heating appreciably before the saturated air in the chamber reached 43.3°C. A hole (24 cm diameter) in the other end of the aluminum case lead to the intake end of a blower (Model 2C889, Dayton Electric Manufacturing Company, Chicago). When the air inside the chamber reached 43.3°C and saturation, the urethane plug was pulled from the end of the aluminum fruit holding case. (At a treatment temperature of 43.3°C, the temperature inside the fruit holding box reached approximately 30°C before the plug was removed.) When the blower motor was turned on, the saturated hot air was pulled through the aluminum fruit holding case. The air that had passed through the fruit travelled through an insulated (2 cm Styrofoam) aluminum duct (2.3 m long by 0.3 m high by 0.15 m wide) along the outside of the chamber and returned to the chamber below the steam outlet.

A platinum resistance thermometer (model S200PD, MINCO Products, Minneapolis) inserted inside a length of hollow cotton wick wetted with distilled water (wet bulb thermometer) and interpreted by a hygro-thermometer (Hygro-M2, General Eastern Instruments Corporation, Watertown, Mass.) provided a signal to a time-proportioning

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modulating circuit (TP Modulator, DRI-STEEM). This signal turned the steam generator on and off. An air speed of 2.5 m/s over the wet bulb thermometer was obtained by placing the tip of the thermometer inside a plastic tube (18 mm inside diameter by 14 cm long) connected to a small fan (Model 606NS, Rotron Manufacturing Company, Woodstock, N.Y.). An additional wet bulb thermometer was constructed of a copper-constantan thermocouple (#36 gauge) sheathed with a hollow cotton wick wetted with distilled water and placed in the plastic tube containing the platinum resistance thermometer. Dry bulb thermocouples were situated inside the fruit holding box and in the larger chamber.

Thermocouples were inserted to the center of grapefruits through glass micropipettes (0.8 mm inside diameter), which guided the thermocouple straight into the fruit. A small drop of hot melt glue (Catalogue No. 212, Emhart Consumer Group, Reading, Pa.) was applied to both ends of the pipette after the thermocouple was inserted to prevent liquids from migrating through the pipette and influencing the temperatures. The junction of the thermocouples protruded through the adhesive at the end of the pipette. The insertion hole on the grapefruit was sealed with 0.15 ml of the glue. The thermocouples were monitored with a datalogger (Model 2285B, John Fluke Manufacturing Company, Everett, Wash.), and data were recorded by a computer. The thermocouples and platinum resistance thermometer were calibrated at temperatures near 43°C with a certified precision quality thermometer (Taylor Scientific Instruments, Arden, N.C.).

Air speed (0.72–0.87 m/s during the tests) was determined by placing an anemometer (#76805, Cenco, Chicago) 6 cm inside the center of the entrance to the aluminum fruit holding case. Air speed was visually recorded immediately after the treatment when air temperatures inside the chamber were approximately 33°C.

Market Quality of Treated Grapefruit. Three types of grapefruit (1: freshly harvested, green; 2: 'degreened' but unprocessed, and 3: fully processed and in cold storage) were tested for their ability to tolerate vapor heat treatments. Degreening grapefruit changes the peel color of early season grapefruit (harvested in October–December in Florida) from green to yellow by holding the green fruit in an atmosphere containing 5–7 ppm ethylene at 29°C and at least 92% RH for up to 96 h (McCormack & Wardowski 1977). Degreening reduces the amount of chlorophyll in the peel but does not alter the sugar or acid content of the pulp. Complete processing of grapefruit involves washing, drying, waxing, and drying again on an automated conveyor system. Grapefruit peel might suffer from the abrasion of washing, the heat of drying, and bruising due to handling, which might be exacerbated by the vapor heat treatment.

Grapefruits were subject to vapor heat at 43.3–43.7°C for 4–5 h and at 46–46.4°C for 3.75 h. Vapor heat treated and untreated fruits were washed, dried with hot air, waxed (StaFresh 705, diluted with water to 10% strength; FMC Corporation, South Charleston, W. Va.), and dried again with a commercial fruit handling system (American Conveyor Corporation, Miami). Grapefruits were stored at 13°C for 30 d, then at 24°C for another 30 d. Observations on the overall appearance of the fruit, numbers of rotted fruit, color, and taste were made weekly. Color was determined in the Judd-Hunter system using a colorimeter calibrated with a white (#05-1456) standard (Pacific Scientific, Colorgard System, Model 05, Silver Spring, Md.). Data resulting from Judd-Hunter measurements are Lh (or L), which measures intensity from 0 (black) to 100 (white); ah (or a), which measures green (negative value) to red (positive value); and bh (or b), which measures blue (negative value) to yellow (positive value). Red-green and yellow-blue paired responses are essentially how the human eye sends responses to the brain (Francis 1980). Taste tests of the juice were performed by personnel of the laboratory. Four replicates of 72 grapefruit per treatment or control were done. Data were analyzed by analysis of variance; means were separated by Fisher's least significant difference test (SAS Institute 1985).

Estimation of Probit 9. 'Marsh' grapefruit (size #36, mean weight = 426.9 ± 80.8 g) were infested with the Caribbean fruit fly in the manner described by Hallman & Sharp (1990). Grapefruit remained in the infestation cage for 1 wk and were held for another 7–10 d at 23°C before treatment so that third instars would be present. Although eggs were reported more resistant to heat (water at 43.3°C) than were third instars (Sharp & Chew 1987), third instars burrow deep into grapefruit (Calkins & Webb 1988) and, therefore, are exposed to less total heat than eggs, which are laid in the grapefruit peel. The grapefruit were randomly separated into five equal lots of 40 fruit each. Each of the four lots was subjected to vapor heat treatment at 43.3–43.7°C for 60, 90, 120, or 150 min. The grapefruit were placed in the fruit chamber on top of horizontal steel rods (3 mm diameter), which prevented contact with other grapefruit or the walls of the chamber; this arrangement optimized heating of the grapefruit to estimate the minimal conditions of temperature and time necessary to achieve probit 9 security (99.9968% mortality) (Baker 1939, Sharp 1989). The remaining lot of grapefruit was not treated and was used to estimate the number of larvae of *A. suspensa* present.

After vapor heat treatment, the five lots of grapefruit were placed on racks at 25°C so that surviving larvae could emerge from the fruit and drop into sand where they were collected and counted (Hallman & Sharp 1990). All emerging larvae were counted as survivors. Five replicates were used. Data were analyzed with the PROBIT procedure (SAS Institute 1985). Probit 9 mortality and 95% FL were estimated as described by Finney (1971).

Table 1. Judd-Hunter color readings after 30 d at 13°C and percentages of rotted fruit after 30 d at 13°C followed by 30 d at 24°C for size #36 freshly picked, degreened 'Marsh' grapefruit subjected to vapor heat treatment

Vapor heat treatment	Mean color reading			Mean % rotted fruit
	ah	bh	Lh	
No treatment	2.89a	43.3a	84.8a	2.9a
43.3–43.7°C, 5 h	2.86a	42.8a	84.4a	2.8a
46–46.4°C, 3.75 h	5.84b	42.1a	81.4a	9.4b

Means followed by same letter not significantly different ($P > 0.05$; Fisher's least significance difference test [SAS Institute 1985]).

Confirmatory Test. More than 100,000 larvae of *A. suspensa* in grapefruits were subjected to vapor heat at 43.3–43.7°C for 4.5 h to ensure that probit 9 security was achieved. Grapefruits were handled in the same manner as described for the preliminary test. Twelve to 25% of the infested grapefruit were not treated and were used to estimate numbers of larvae present in the treated grapefruit.

Results

Market Quality of Treated Grapefruit. In three tests performed on separate dates, vapor heat treatment at 46–46.4°C for 3.75 h of degreened, freshly picked grapefruit that had received no other processing resulted in darkening of the oil glands similar to chilling injury (Smoot et al. 1971). This symptom appeared a few days after treatment. The darkening was quantified by significantly higher ah (red) reading in the Judd-Hunter system compared with untreated fruits or grapefruits treated at 43.3–43.7°C for 5 h (Table 1). More grapefruits that were treated with vapor heat at 46–46.4°C rotted after 60 d compared with untreated grapefruits or those treated at 43.3–43.7°C (Table 1).

Only one replicate of freshly harvested, green grapefruit was subjected to both vapor heat treatments, and the response was similar to degreened, unprocessed grapefruit. Darkening of the oil glands occurred in grapefruit treated at 46–46.4°C, but not in those treated at 43.3–43.7°C.

Internal appearance and aroma of vapor heat treated and untreated fruits sliced in half did not differ; the taste of juices squeezed from the grapefruit halves did not differ.

Grapefruit that had been completely processed and stored at 13°C for 2–4 wk and then subjected to vapor heat at 43.3–43.7°C for 4 h or 46–46.4°C for 3.75 h showed marked darkening of oil glands compared with the untreated controls.

Estimation of Probit 9. A total of 1,276 larvae of *A. suspensa* was recovered from the controls of the five replicates (Table 2). Probit analysis of mortality using the logarithm (base 10) of time yielded a χ^2 probability of 0.27 (y intercept = −7.32, variance of the intercept = 0.131, slope = 6.80, SE of slope = 0.22, variance of the slope = 0.0000166, covariance = −0.00142). Probit 9 was estimated to be 252.0 min (95% FL 235.3 to 272.4 min).

Table 2. Results of vapor heat treatment (43.3–43.7°C) of grapefruit infested with *A. suspensa* larvae (mostly third instars)

Replicate	Estimated no. larvae/treatment	No. larvae killed at each treatment time (min)			
		60	90	120	150
1	508	155	394	495	505
2	154	0[a]	107	153	151
3	232	151	227	228	228
4	157	0[a]	129	157	157
5	225	0[a]	192	206	225
Total	1,276	306	1,049	1,239	1,266

[a] Data not used in probit analysis; estimated no. larvae killed <0.

Confirmatory Test. An estimated total of 103,470 *A. suspensa* larvae (mostly third instars) was subjected to vapor heat treatment at 43.3–43.7°C for 4.5 h inside of 6,321 size #36 'Marsh' grapefruit; three survived. The first surviving larva, located after 28,173 estimated larvae were killed, became an adult male that was normal in appearance. The second and third larvae appeared after an estimated 86,674 and 88,203 larvae were killed and died as pupae that looked normal in appearance.

To raise center temperatures of #36 grapefruit from 24–26°C to 43.3°C required 220–259 min; center temperatures remained at ≥43.3°C for 11–50 min.

Discussion

R. Nguyen (personal communication) found no larvae of *A. suspensa* in 400,000 grapefruit collected from commercial groves in Florida from 1984 to 1988. During January and February 1984, five *A. suspensa* larvae were recovered from 14,000 grapefruit collected from residential yards (R. Nguyen, personal communication). Grapefruits from residential yards are not shipped commercially. Landolt et al. (1984) argued that requiring probit 9 security, which would necessitate killing 100% of 93,613 larvae tested to attain 95% confidence (Couey & Chew 1986), was excessive in a fruit that had a very low infestation rate by a fruit fly. Florida grapefruits shipped to Japan are often treated with a cold quarantine procedure that ensures less than probit 9 security depending on the adult density of *A. suspensa* in the groves (Ismail 1989). The same reasoning could be applied to vapor heat treatments.

To achieve probit 9 security with 95% confidence, the length of the vapor heat treatment under the condition of this study would have to be increased. In any case, a vapor heat treatment should not be based on the amount of time fruits are subjeted to the treatment, but the amount of time the center of the fruits remain at a certain temperature. The rate at which fruits heat when subjected to vapor heat is affected by fruit size, air speed, size of the entire fruit load, and arrangement of the

fruits within the load (Baird & Gaffney 1976, Furusawa et al. 1984, Chau et al. 1985). During our study, 220–259 min were required to raise center temperatures of #36 grapefruit from 24–26°C to 43.3°C; center temperatures remained at ≥43.3°C for 11–50 min. We recommend that size #36 grapefruit infested with *A. suspensa* be subjected to vapor heat at 43.3–43.7°C until the centers of fruits in areas of the treatment room likely to be coolest reach 43.3°C for 50 min. The location of cool spots will depend on each particular treatment facility and will have to be determined when the facility is certified.

Vapor heat requirements for treating grapefruit infested with *A. ludens* at 43.3°C include an 8-h period to raise temperatures at the center of the fruit to 43.3°C followed by 6 h at that temperature. In our research, temperatures at the center of #36 grapefruit reached 43.3°C in 3.67–4.32 h. Earlier vapor heat machinery did not pull air through the fruit load but circulated air around the load relying on turbulence and diffusion to move the saturated hot air into the interior of the fruit load (Mackie 1931). Sinclair & Lindgren (1955) raised temperatures at the center of grapefruit (size not given) from 22.2 to 43.3°C in 5 h with vapor heat at 43.3°C.

The reason for a 6-h treatment period for grapefruits infested with *A. ludens* once center temperatures had reached 43.3°C is not apparent. Data from studies by Sharp (1988) with hot-water immersion indicate that *A. ludens* may be slightly more resistant to heat than *A. suspensa,* but not enough to warrant a 6-h treatment after temperatures at the center of the grapefruits had reached 43.3°C.

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References Cited

Baird, C. D. & J. J. Gaffney. 1976. A numerical procedure for calculating heat transfer in bulk loads of fruits or vegetables. ASHRAE (Am. Soc. of Heating, Refrigeration, and Airconditioning Engineers) Trans. 82: 525–540.

Baker, A. C. 1939. The basis for treatment of products where fruit flies are involved as a condition for entry into the United States. USDA Circular 551.

Calkins, C. O. & J. C. Webb. 1988. Temporal and seasonal differences in movement of the Caribbean fruit fly larvae in grapefruit and the relationship to detection by acoustics. Fla. Entomol. 71: 409–416.

Chau, K. V., J. J. Gaffney, C. D. Baird & G. A. Church II. 1985. Resistance to air flow of oranges in bulk and in cartons. Trans. ASAE (Am. Soc. Agric. Eng.) 28: 2083–2088.

Couey, H. M. & V. Chew. 1986. Confidence limits and sample size in quarantine research. J. Econ. Entomol. 79: 887–890.

Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge University Press, Cambridge, England.

Francis, F. J. 1980. Color quality evaluation of horticultural crops. HortScience 15: 58–59.

Furusawa, K., T. Sugimoto & T. Gaja. 1984. Effectiveness of the vapor heat treatment against the melon fly, *Dacus cucurbitae* Coquillett, in eggplant and fruit tolerance to the treatment. Res. Bull. Plant Prot. Serv. Jpn. 20: 17–24.

Hallman, G. J. & J. L. Sharp. 1990. Hot-water immersion quarantine treatment for carambolas infested with the Caribbean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 83: 1471–1474.

Ismail, M. 1989. Cold treatment: a successful quarantine method for Florida grapefruit shipped to Japan. Packinghouse Newsletter 158: 1–2.

Landolt, P. J., D. L. Chambers & V. Chew. 1984. Alternative to the use of Probit 9 mortality as a criterion for quarantine treatments of fruit fly (Diptera: Tephritidae)-infested fruit. J. Econ. Entomol 77: 285–287.

Mackie, D. B. 1931. Heat treatments of California fruits from the standpoint of compatibility of the Florida process. Mon. Bull. Dep. Agric. Calif. 20: 211–218.

McCormack, A. A. & W. F. Wardowski. 1977. Degreening Florida citrus fruit: procedures and physiology. Proc. Int. Soc. Citric. 1: 211–214.

SAS Institute. 1985. SAS user's guide: statistics, version 5 ed. Cary, N.C.

Sharp, J. L. 1988. Status of hot water immersion quarantine treatment for Tephritidae immatures in mangos. Proc. Fla. State Hortic. Soc. 101: 195–197.

1989. Research on the hot water dip and viable alternatives, pp. 35–38. *In* Report on seminar on quarantine treatments for fresh tropical fruits entering the United States of America, July 10–14, 1989, Mazatlan, Mexico. USDA, Office of International Cooperation and Development.

Sharp, J. L. & V. Chew. 1987. Time/mortality relationships for *Anastrepha suspensa* (Diptera: Tephritidae) eggs and larvae submerged in hot water. J. Econ. Entomol. 80: 646–649.

Sinclair, W. B. & D. L. Lindgren. 1955. Vapor heat sterilization of California citrus and avocado fruits against fruit-fly insects. J. Econ. Entomol. 48: 133–138.

Smoot, J. J., L. G. Houck & H. B. Johnson. 1971. Market diseases of citrus and other subtropical fruits. USDA Handbook 398.

USDA-APHIS. 1985. Plant protection and quarantine manual, sec. III, part 9 and sec. VI-T106.

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