RADIO FREQUENCY PULSE APPLICATION FOR HEATING UNIFORMITY IN POSTHARVEST CODLING MOTH (LEPIDOPTERA: TORTRICIDAE) CONTROL OF FRESH APPLES (MALUS DOMESTICA BORKH.)

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ABSTRACT

A radio frequency (RF) treatment, with a pulse mode to increase heating uniformity, was examined as a potential quarantine treatment against fifth instars of the codling moth, Cydia pomonella L. (Lepidoptera: Tortricidae), in apples, Malus sylvestris L. var. domestica (Borkh.) Mansf., intended for export to Japan. Apples were exposed in water baths to 27.12-MHz RF energy at 12 kW with a pulse mode of 30 s-on/30 s-off for different durations. The temperature uniformity was determined by measurements at 12 sites evenly spaced at two depths in the fruit. The most promising exposure time was 29 min. The efficacy of the treatment with the pulse mode was examined against the codling moth larvae by using exposures between 27 and 30 min and up to 30 min of holding time in bath after treatment. The treatment that killed all larvae with the least amount of energy was the 29-min exposure with 50-min holding time. This treatment was appraised for fruit quality in "Delicious" apples. Some injury was observed when treated fruits were removed from 1C storage after 30 days. When these fruits were held at 25C for 1 week, all were too damaged to evaluate for fruit quality. Although pulse mode increased heating uniformity in the fruit, the thermal

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requirements to control codling moth larvae may exceed the injury threshold of the fruits.

INTRODUCTION

Quarantine regulations prevent the introduction of specific pests on imported agricultural commodities. Postharvest treatments have been developed to control these pests, and to maintain international commerce, Japan requires a two-component treatment involving cold storage and methyl bromide fumigation to control codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), in fresh apples, *Malus sylvestris* L. var. *domestica* (Borkh.) Mansf. (Hansen *et al.* 2000). However, the future use of methyl bromide is questionable because of environmental and health concerns (Anonymous 1990, 1995). The potential prohibition of methyl bromide has led to investigations on alternative approaches.

Thermal treatments are being evaluated as replacements for methyl bromide fumigation to control codling moth larvae. Jones and Waddell (1997), using direct immersion into hot water, found the third instar to be the most heat-resistant. Other thermal studies using dry larvae reported the fifth instar as the most thermal-tolerant stage (Yokoyama *et al.* 1991; Wang *et al.* 2002b). Further studies examined the kinetic relationship of insect mortality in the fifth instar with treatment parameters (Wang *et al.* 2002a; Hansen *et al.* 2004b). Various heat treatments, alone or in combination with other physical treatments, have been proposed as replacements to methyl bromide fumigation (Neven 1994; Neven and Rehfield 1995; Neven *et al.* 1996).

A unique heating method is to use radio frequency (RF) energy, where heat is generated from internal resistance when subjected to very rapid alterations of polarity (Headlee and Jobbins 1936). This technology is appealing for postharvest treatments because the heating is fast and linear with time, energy can penetrate deep into the commodity, there are no chemical residues, and the process has minimal impact on the environment (Tang *et al.* 2000). RF treatments have been examined for codling moth control in apples, but have suffered because of the lack of uniform temperatures within the fruits even though the fruits were moving within a water-filled container and then held for durations in a hot water bath (Birla *et al.* 2004; Hansen *et al.* 2004a, 2006) A possible solution to nonuniform heating is to use the pulse mode on the RF unit where energy is applied in short bursts, followed by an inactive period to allow for heat dispersal.

The objectives of our study were to develop procedures that resulted in uniform fruit heating, to test this treatment for efficacy against fifth instar codling moth, and to evaluate the treatment effects on fruit quality after cold storage.

MATERIALS AND METHODS

Experimental Unit

The RF unit was the same as in previous studies (Hansen *et al.* 2004a, 2006). Tests were conducted in a 12-kW, 27-MHz pilot-scale RF heater/dryer with an E-200 control panel (Strayfield Fastran, Strayfield International Limited, Wokingham, U.K.). The interior of the treatment cavity consisted of two 104.8×79.4 cm electrodes, in which the bottom one was stationary whereas the top one could be elevated from 200 to 400 mm. The power coupled in the sample could be tuned slightly by adjusting the lengths of three paired 7.6-cm-wide interconnected parallel plates above the top electrode (the top two plates were 76-cm long and the bottom plate was 66-cm long) so that the functional length could be manipulated. A high-energy RF generator induced energy between the electrodes. Because the unit was rated at 12 kW, the maximum efficiency of 2 amp was controlled by lowering or raising the top electrode. The pulse mode on the unit controlled both the duration of exposures and time of inactivity.

Forty fruits were treated while floating and moving in a 25-gal tank within the RF unit. The tank was a round low-density polyethylene container (Konnex Plastics, La Mirada, CA), 13-in. high with an upper 26.5-in. inside diameter (i.d.) and a lower 24-in. i.d. polyvinyl chloride (PVC) piping connected the tank to a two-speed, 1.5 hp Century Lasar Pool/Spa Motor Pump (Aqua-flo, Inc., Chino, CA) located outside the unit. The piping consisted of in- and out-flow lines, attached to the side of the tank, and a nozzle below the surface to direct the water flow. Fruits circulated while floating on the surface at 5–7 cycles/min. Water conductivity was adjusted to about 600 μ s by about 5 mL of calcium chloride. Internal fruit temperatures (1- and 2-cm deep) were measured immediately after treatment by using a UMI 8 channel conditioner with 0.12C resolution (model FOT-L/CRM/3m) fiber optic transducers (FISO Technologies, Inc., Sainte-Foy, Québec, Canada). Data acquisition was performed using the FISO Commander software (FISO Technologies, Inc.) and temperatures were measured every 0.05 s.

Uniform Heating Tests

Fruits for the uniformity studies were size 100 "Golden Delicious" and "Delicious" apples previously held in 4C regular air storage. The fruits were initially treated at \approx 21C for both fruits and water. Two pulse modes were used: (1) 30 s-on/20 s-off; and (2) 30 s-on/30 s-off. The total RF exposure time (time within the RF unit) was between 27 and 30 min. Fruits were also held in the same tank after RF treatment for set holding periods. The fruit temperature was measured after treatment by using a digital thermometer (model HH501DK,

Omega Engineering, Inc., Stamford, CT) equipped with a k-type thermocouple, and the response time was listed as 0.8 s. For each test, 24 temperature measurements were taken on a fruit at depths of 1 and 2 cm along four perpendicular longitudinal axes at the equator of the fruit and at the midpoint between the equator and each pole (12 locations with two depths at each location). Core temperatures were not taken because of the uncertainty of the locations of the internal cavities. Pulp temperatures from treatments needed to average 50C in the shortest period with the smallest range of temperatures. When 50C was reached, the test was repeated thrice.

Efficacy

The efficacy tests used codling moth larvae from a laboratory colony maintained on a soy-wheat germ-starch artificial diet at $\simeq 27C$, 40–58% relative humidity (RH), with a 16:8 h light : dark photoperiod (Toba and Howell 1991). To infest, late fourth to early fifth instars were removed from the artificial diet and four larvae were placed on the stem end of each apple, either "Gala" or "Golden Delicious." Late fifth instars were excluded because they would form cocoons for pupation instead of feeding. The infested apples were held overnight (12–18 h) at \approx 24C, 60–70% RH, with a 16:8 h light : dark photoperiod. Infested fruits were subjected from 27 to 30 min of 30 s-on/30 soff exposures, plus holding times up to 40 min, followed by hydrocooling at \simeq 20C. Replication varied with time of RF exposure based on the number of survivors: four replicates for 27 min, two replicates for 28 min, eight replicates for 29 min and two replicates for 30 min. Pulp temperatures were measured the same as in the uniform heating tests. The treated fruits were evaluated the next day by dissecting them and recording the number of live and dead larvae observed.

Fruit Quality

Size 100 Washington Extra Fancy apples (cultivar "Delicious") freshly harvested (<2 months) and obtained locally were used for fruit quality tests. There were four replicates, each with 40 fruits, including the control. Fruits were treated at the shortest time period among the efficacy studies: 30 s-on/ 30 s-off pulse exposure for 29 min plus 5 min in holding tank, followed by hydrocooling (\approx 20C), then placing in cold storage (1C) after drying. Quality parameters were evaluated twice on 20 fruits, either immediately after 1 month regular atmosphere (RA) cold storage or after an additional week at room temperature (\approx 25C).

The quality factors evaluated were external and internal color, firmness, soluble solids, titratable acidity and external and internal disorders. Color was determined using a color meter (The Color Machine, Pacific Scientific, Silver

Springs, MD) using the Hunter L^* , a^* , b^* system and calculated hue values (Hunter and Harold 1987). Two evaluations were made on each fruit for external color and one evaluation was made for internal color. Firmness was determined at two locations per fruit with a texture analyzer (TA-XT2, Texture Technologies, Scarsdale, NY) equipped with an 11.1-mm probe and values were reported in Newtons (N). Soluble solids and titratable acidity were determined from an aliquot of expressed juice of a longitudinal slice from each of 10 fruit. An Abbe-type refractometer with a sucrose scale calibrated at 20C was used to determine soluble solids. Titratable acidity was measured with a titrator (TTT 85, Radiometer, Copenhagen, Denmark). Acids were titrated to pH 8.2 with 0.1 N NaOH and expressed as p% malic acid. Disorders (internal browning and bruising) were evaluated by two laboratory personnel and expressed as affected fruit.

Statistical Analyses

Means and SDs were calculated using an Excel 2002 (Microsoft Corp., Redmond, WA) spreadsheet. The fruit quality parameter data were analyzed using SAS (SAS Institute, Cary, NC). PROC MEANS was used for univariate statistics, and nonparametric tests were used to determine significant differences by first arranging data by PROC RANK and then performing PROC general linear model (GLM). Student's *t* tests and analysis of variance (ANOVA) were carried out using PROC GLM. This approach was equivalent to a Wilcoxon rank sum test for two samples and the Kruskal–Wallis *k*-sample test for more than two samples (Zolman 1993).

RESULTS AND DISCUSSION

Uniform Heating Tests

A necessary requirement for a heat treatment is to have a sufficient pulp temperature with the least amount of variation between the highest and lowest temperatures in the fruit. In the preliminary test with the pulse mode set at 30 s-on/20 s-off, the range among the pulp temperatures within a fruit was \geq 4C, too high compared to their respective mean temperatures (Fig. 1A). The disparity in extreme temperatures decreased when the off phase was increased (mode = 30 s-on/30 s-off) (Fig. 1B). The 27-min RF exposure resulted near the intended efficacious temperature of 50C (Hansen *et al.*, 2006) with a range <3C. When the exposure was extended to 29 min and repeated four times, the mean temperature was >51.5C for each replicate and the temperature ranges were between 3.6 and 6.0C (Table 1); the final water temperature (mean ± standard error of the mean) was 51.8 ± 0.4C. Temperatures were



FIG. 1. COMPARISONS OF HEATING UNIFORMITY IN APPLE OF TEMPERATURES MEASURED AT 12 EVENLY SPACED SITES AND AT DEPTHS OF 1 AND 2 cm AFTER RADIO FREQUENCY (27.12 MHz) EXPOSURE AT 12 kW FOR DIFFERENT DURATIONS (min) (A) Pulsed mode of 30 s-on/20 s-off; (B) pulsed mode of 30 s-on/30 s-off.

significantly different between the two depths (F = 62.6; df = 4, 91; P < 0.01) by using ANOVA. Comparisons showed few significant differences of pulp temperatures at either depth between those sites on the stem end (above the water bath) and those near the calyx end (completely submerged) (Table 2). Evidently, floating in the container during RF treatment did not cause differential heating in the outer portions of the fruits.

Holding the fruits in the treatment container for 5 min after the 29-min exposure resulted in mean temperatures near 51.0C with ranges <4C (Table 1). The temperatures after 5 min of holding were significantly less than those

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TABLE 1.

PULP TEMPERATURES OF 12 SITES AT DEPTHS OF 1 AND 2 cm (n = 24) IN "DELICIOUS" APPLES AFTER TREATMENT OF RADIO FREQUENCY (27.12 MHz) EXPOSURE AT 12 kW FOR 29 min WITH A PULSE MODE OF 30 s-ON/30 s-OFF, FOLLOWED BY A HOLDING TIME OF 0 OR 5 min

Holding time (min)	Replicate	Temperature (C)		
		Mean	SD	Range
0	1	51.7	1.1	4.1
	2	52.6	1.1	3.7
	3	52.6	0.9	3.5
	4	53.6	1.6	6.0
5	1	51.2	1.0	3.3
	2	50.9	0.7	2.7
	3	51.1	0.9	3.1

TABLE 2.

MEAN PULP TEMPERATURES OF FOUR SITES BY DEPTH (1 AND 2 cm) AND POSITION IN TWO APPLE CULTIVARS AFTER TREATMENT OF RADIO FREQUENCY (27.12 MHz) EXPOSURE AT 12 kW FOR 29 min WITH A PULSE MODE OF 30 s-ON/30 s-OFF

Replicate	Depth (cm)	Mean temperature (C) (position on fruit)		F value	Р
		Stem end	Calyx end		
"Golden Del	icious"				
1	1	52.8	52.0	1.25	ns
1	2	55.7	54.6	6.14	*
2	1	51.0	50.6	0.18	ns
2	2	53.3	52.3	4.48	ns
3	1	51.9	51.2	0.84	ns
3	2	54.6	53.0	13.65	**
4	1	51.0	50.3	1.45	ns
4	2	52.3	51.5	3.99	ns
"Delicious"					
1	1	51.0	50.6	0.43	ns
1	2	52.8	52.0	2.75	ns
2	1	51.1	52.4	6.42	*
2	2	53.1	53.0	0.06	ns
3	1	51.8	51.8	0.06	ns
3	2	53.6	52.7	7.39	*
4	1	52.3	52.5	0.02	ns
4	2	54.4	54.0	0.35	ns

Significant differences between the stem and calyx end temperatures were determined by Student's *t*-test (df = 1, 7).

* Significant at P < 0.05. ** Significant at P < 0.01.

ns, not significant.

without holding (F = 16.15; df = 1,6; P < 0.05). This holding in the treatment container after RF exposures allows the heat in different areas of the fruit to dissipate and become more equal throughout, a major step for heating uniformity. Longer holdings would aid in temperature equalizations.

Our methodology is an improvement over previous attempts in using RF treatments of apples. Birla *et al.* (2004) reported an average SD of 2.2C for seven fruits with a mean pulp temperature of 51.1C from six locations after 5.5 min of continuous RF treatment. Hansen *et al.* (2004a) measured the shortest range of pulp temperature to be 7C, with a mean of 45.2C for continuous RF exposures of 10 min. However, the pulp temperatures in this study were significantly higher at the deeper depth. Although core temperatures were not measured, even higher temperatures would be expected.

Efficacy

Dissimilar larval mortality was recorded when infested apples had different RF exposures and then held for various times (Fig. 2). The longer the RF exposure and the greater the holding time for each of these exposures, the greater the mortality. The 27-min exposure required a 30-min holding time, whereas the 29-min exposure had 100% mortality with 5 min of holding time. Survivors were found at 30-min exposure and no holding time. As discussed in the previous section, holding time provides for a more equal distribution of heat within the fruit. The pulp temperatures of treated uninfested-control "Delicious" apples were not statistically different (P > 0.05) from those of



FIG. 2. LARVAL MORTALITY OF CODLING MOTH TREATED AT DIFFERENT RADIO FREQUENCY (27.12 MHz) EXPOSURES AT 12 kW (SYMBOLS) AT A PULSE MODE OF 30 s-ON/30 s-OFF AND HELD AT SPECIFIC HOLDING TIMES

"Golden Delicious" used in the uniformity tests previously conducted, and there were few differences between the top and bottom spheres of the fruits, regardless of depth, which was also similar to the observations with "Golden Delicious" (Table 2). However, ANOVAs indicated that temperatures were significantly different between the two depths (F = 35.4; df = 4, 91; P < 0.01).

A treatment temperature of \approx 50C seems to be the most practical against codling moths in apples. Neven *et al.* (1996) predicted 99% mortality of fifth instar codling moths in fruits when using vapor heat (saturated moist air) at 48.5C for 73 min. Neven (1994) reported 72% mortality in fifth instar codling moths alone after 30 min in 45C hot air. Hansen *et al.* (2004a) needed 20 min of holding time at a pulp temperature of 50C to obtain 100% mortality against fifth instar codling moths in apples. Hansen *et al.* (2006) observed 100% kill of fifth instar codling moths in apples by using a 205-s RF exposure, followed by 40 min in a 50C water bath.

Fruit Quality

Fruits for the quality studies were subjected to the least adverse efficacious treatment, 29-min exposure followed by 5-min holding. All treated fruits stored for 30 days (1C) were darker than control fruits after similar storage (Table 3). Treated fruits displayed scald, had signs of peel burning and displayed internal browning (Fig. 3), none which were observed in the untreated control fruits. Regardless, all fruits treated with RF displayed internal browning after only a short storage period.

Because of the trend of increased temperatures with increased depth, the fruit cores can be assumed to be the hottest part of the fruits. Hansen *et al.* (2006) reported significant damage in all apples treated with RF and then held in hot water for sufficient times to kill fifth instar codling moth. Furthermore, treated fruits tended to be firmer than the control fruits, a trait contrary to that observed in other RF-treated apples (Hansen *et al.* 2006).

Treated fruits that were kept a week at 25C after a month at 1C storage were too injured to be measured for quality. This is similar to the observation earlier that none of the RF treated apples survived RA 1C storage for 60 days. To allow for routine commercial transactions in exporting apples to Japan, adequate fruit quality must be sustained for at least 2 months (Aegerter and Folwell 2000). Progress is needed to extend the shelf life of heated fruits before RF can be considered as a quarantine treatment.

CONCLUSIONS

We have made progress in increasing heating uniformity, which impacts treatment efficacy, in batches of fruits under conditions that kill all infesting TABLE 3.

FRUIT QUALITY PARAMETERS OF "DELICIOUS" APPLES
TREATED WITH RADIO FREQUENCY (27.12 MHz)
EXPOSURE AT 12 kW FOR 29 min WITH A PULSE MODE OF
30 s-ON/30 s-OFF AND 5-min HOLDING IN A 50C BATH,
THEN HELD IN 1C STORAGE FOR 1 MONTH

Quality Parameters	Control	Treated		
		Mean	SEM	95% CI
Whole fruit				
Firmness (N)	59.62	65.85	0.81	2.58
Soluble solids (%)	13.40	11.83	0.19	0.60
Titratable acidity (%)	0.19	0.16	0.00	0.01
Peel				
Hunter L	23.30	25.95	0.83	2.63
Hunter a	19.59	19.07	0.58	1.83
Hunter b	5.84	6.29	0.41	1.29
Hue	16.70	18.32	0.83	2.63
Scald (%)	0	100	0	0
Finish	1	2.13	0.13	0.40
Burn (%)	0	27.5	2.3	10.27
Flesh				
Hunter L	68.77	69.46	0.83	2.65
Hunter a	-0.46	-1.75	0.22	0.70
Hunter b	21.33	21.17	0.29	0.93
Hue	91.23	94.25	0.56	1.77
Internal browning (%)	0	15.6	6.0	1.90

Four treated replicates, with 40 fruits in each replicate including the control.

codling moth larvae. Unfortunately, maintaining long-term fruit quality is still elusive. Like other temperate fruits and vegetables, the respiration rate of apples increase two to three times for every 10C rise in pulp temperature (Hardenburg *et al.* 1986), so that at treatment temperatures (\approx 50C) the estimated carbon dioxide production may be 120–200 mg/kg·h, which indicates premature aging of fruit tissue. Quality retention would be improved if treatment occurred at time of harvest, but marketing decisions to Japan would have to be committed then, which would severely reduce flexibility to adjust to changing prices. A technical breakthrough that shields the fruit tissues from the adverse thermal effects is needed to promote RF postharvest treatments.

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FIG. 3. INTERNAL BROWNING OF "DELICIOUS" APPLE TREATED WITH RADIO FREQUENCY (27.12 MHz) EXPOSURE AT 12 kW FOR 29 min WITH A PULSE MODE OF 30 s-ON/30 s-OFF AND 5-min HOLDING IN A 50C BATH, AND THEN HELD AT 25C FOR SEVERAL DAYS

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