Condition of Curry Leaves Exposed to Quarantine Treatments of Cold, Gamma Irradiation, or Methyl Bromide Fumigation

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Abstract. In separate treatments, leaves of the curry leaf tree [*Murraya koenigii* (L.) Sprengel] were subjected to 15 days at 1 °C, to gamma irradiation from a ⁶⁰Co source at dosages from 100 to 1000 Gy, or to fumigation with methyl bromide from 16 to 64 g·m⁻³. Cold-treated leaves retained their fresh appearance with minimal weight loss when stored in sealed plastic bags; although color intensity of the underside of leaves was measurably reduced, the change was not visually perceptible. Similarly, irradiation was associated with a darkening of leaves and a loss of color intensity, but these changes were not visibly apparent even at the highest dosage. Methyl bromide fumigation, however, greatly increased susceptibility of leaves to postharvest decay. Cold or gamma irradiation treatments should be tolerated by this commodity when treated for pests such as the Asian citrus psyllid (*Diaphorina citri* Kuwayama) prior to export.

The curry leaf tree, a small deciduous tree in the family Rutaceae, provides one of the aromatic leafy spices characterizing Indian cuisine. Native to forests along the foot of the southern Himalayas, the plant has spread as far as northern Thailand and is widely cultivated in Malaysia. Growers in South Florida also produce this crop and ship it during the winter to markets in Los Angeles and other areas in the United States where large populations of Asian descent have congregated. Although indigenous Florida insects such as armored scales can be found associated with this plant, infested leaves would be culled before shipment and have not posed a serious threat to interstate commerce.

In June 1998, the Asian citrus psyllid was discovered in Martin, Palm Beach, and Broward Counties, Fla. (Halbert, 1998). This insect had been intercepted previously by the Animal Plant Health Inspection Service (APHIS) of the U.S. Dept. of Agriculture (USDA) on rutaceous plants from Asia, primarily on species of *Murraya* but also on citrus. Although its recent introduction may have resulted from produce illegally transported from Asia, the psyllid also may have entered the United States by natural spread through Central America and the Caribbean

from a naturalized population in South America. The insect causes minimal damage to plants unless populations are high, and species of *Citrus* and *Murraya* are common or preferred hosts. A much greater risk to agriculture is the threat of transmission by the psyllid of citrus greening disease, thought to be caused by a fastidious phloem-limited bacterium (*Liberobacter* sp.). Infected plants become stunted and sparsely foliated with off-season bloom, fruit drop, and twig die-back (Aubert, 1987). This threat to citrus has prompted officials in California to scrutinize imports of rutaceous plant material from Florida. Since its introduction, the Asian citrus psyllid has now spread into agricultural regions of Dade County, in the southern tip of Florida, where the curry leaf tree is grown.

Current law authorizes cold treatment for produce entering the United States with a specific treatment schedule for fruit held at 32 to 35 °F (0 to 1.67 °C) (USDA, 1998). Carambolas (*Averrhoa carambola* L.) shipped from Florida to California may be treated for 15 d at 1.1 °C to kill Caribbean fruit flies [*Anastrepha suspensa* (Loew.)] (Gould and Sharp, 1990), and a similar treatment could be used for lychees (*Litchi chinensis* Sonn.) (McGuire, 1997). Alternatively, the U.S. Food and Drug Administration has approved the use of ionizing radiation at ≤1000 Gy for treatment of foodstuffs (Young and Bowen, 1986). With the proposal by the U.S. government to allow continued use of methyl bromide (MB) fumigation for quarantine eradication of pests in commodities for export (Browner, 1999), this treatment is also available for consideration. Except for this and other specified critical uses, however, the manufacture or importation of MB is to be phased out completely in 2005, although exporters of many crops, such as apples (*Malus domestica* Borkh.) and cher-

ries (*Prunus avium* L.), prefer its cost and timeliness to other alternatives.

The objective of this work was to evaluate the condition of curry leaves treated with cold, gamma irradiation, or methyl bromide fumigation as possible treatments against the Asian citrus psyllid.

Materials and Methods

Cold treatment. Leaves were harvested from mature trees ≈2–3 m in height from five commercial sites in Dade County, Fla.; each site represented an experimental replication. From each replication, 25 leaves were selected, and two leaflets on each leaf were marked and initially evaluated for color characteristics with a Minolta CR-200 chroma meter (Minolta Corp., Ramsey, N.J.) calibrated to a standard white reflective plate and recording in the lightness (L*), chroma (C*), and hue angle (h°) color system. For L*, 100 is white and 0 is black; for C*, 100 is most intense and 0 is least intense; and for h°, 0° is red-purple, 90° is yellow, 180° is bluish-green, and 270° is blue. Measurements of both the upper and lower leaf surface were taken across an area about 50 mm² with diffuse illumination at a viewing angle of 0° under Commission Internationale de l'Eclairage (CIE) illuminant C conditions (McGuire, 1992). The 25 leaves were weighed as a group, sealed with a damp paper towel inside Ziplock® freezer bags (Dow Brands L.P.; Indianapolis, Ind.) of 18 × 20 cm, then placed at 1 °C for 15 d.

After cold storage, leaves were weighed again, and marked leaflets were evaluated for color of both surfaces as noted above. The percentages of decay and chilling injury were rated using a 12-point visual acuity scale (Horsfall and Barratt, 1945). Decay was evidenced by the development of brown to black surface lesions with or without signs of sporulation by the pathogen and chilling injury by a bronze discoloration and/or local drying of tissues. Leaves in sealed bags were held at 24 °C for 72 h, then decay and injury were reevaluated.

Irradiation treatment. As part of the same harvests noted above, leaves were obtained from five sites, which represented experimental replications. From each site, 275 leaves were removed from stems and randomly divided into 11 treatments of 25 leaves each. A nonirradiated control was set aside, and the other 10 groups were irradiated with a ⁶⁰Co source of gamma radiation at a rate of 1.35 Gy·s⁻¹ to yield an estimated absorbed dose of 100 to 1000 Gy in intervals of 100 Gy. The Gammacell model 220 irradiator (Atomic Energy of Canada, Ottawa), its calibration, and principle of operation have been described by von Windeguth (1986). Leaves of each treatment were subsequently weighed as a group of 25, then sealed with a damp paper towel inside Ziplock® freezer bags.

After 3 d of storage at 7 °C (conditions specified by packers as representing those during transport to market), the leaves were weighed again and rated for percentages of surface decay and injury as noted above. Two

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leaflets per leaf were marked, and the color of the upper and lower surfaces was examined. Leaves in sealed bags were held at 24 °C for 72 h, then color of marked leaflets and weight loss were evaluated for a second time; leaves were also reexamined for decay and injury.

Methyl bromide fumigation. Separate from the above tests, curry leaves were subjected to fumigation with MB. From each of three sites (corresponding to three replications), 100 leaves were collected, removed from stems, and randomized into four treatments, each consisting of 25 leaves. A nonfumigated control was set aside, weighed, and two leaflets on each leaf were marked for an immediate color evaluation prior to bagging with a damp paper towel inside a sealed Ziplock® freezer bag. The other leaves were fumigated with MB at either 16, 32, or 64 g·m⁻³ over 2 h at 24 °C in gallon-sized (3.8 L) wide-mouthed glass jars with Teflon® lids that contained a septum for introduction of gas by syringe. The fumigated leaves were aerated for 20 min, then weighed as a treatment before being placed with a damp paper towel inside sealed bags. Leaves were stored for 3 d at 7 °C.

After storage, leaves were weighed and rated for decay and injury as noted above. Two leaflets per leaf were marked, and the color of the upper and lower surfaces was examined. Leaves in sealed bags were held at 24 °C for 72 h, then color, injury, and decay were reexamined.

Data analysis. Data were analyzed separately for the responses of curry leaves to cold, irradiation, and fumigation. Leaf color after the 1 °C cold treatment was compared with color immediately after harvest, and data were subjected to an analysis of variance (PROC ANOVA) using the SAS statistical software package (SAS Institute, 1985) with mean separation by Dunnett's one-tailed *t* test. The effect of storage at 7 °C for 3 d was similarly compared for control leaves in the irradiation test. Data from the irradiation and fumigation tests were analyzed with PROC REG in SAS. After each analysis, Horsfall/Barratt rating values were converted to mean percentages for estimation of percentage of surface area decayed or percentage of surface area injured.

Results

Cold treatment. Weight loss, which averaged 0.68%, was minimal for curry leaves sealed within plastic bags over the 15 d of cold treatment. After cold treatment, the color values of the upper leaf surface were not significantly different from those of leaves initially measured at harvest (Table 1). Only color intensity, C*, of the lower leaf surface was significantly affected; however, changes in color were not visually perceptible. No decay or chilling injury was seen after cold storage or a further 72 h of storage at room temperature.

Irradiation treatment. Weight loss from irradiated curry leaves averaged 2.28% after 3 d at 7 °C and was unaffected by dosage (*P* = 0.857). After an additional 3 d at room temperature, however, an inverse relationship

Table 1. Color of curry leaves after quarantine treatments of cold, gamma irradiation, and methyl bromide fumigation.

Treatment	Leaf color ^z					
	Upper surface			Lower surface		
	L*	C*	h°	L*	C*	h°
<i>Cold^b</i>						
At harvest	36.1 a	18.7 a	131.1 a	51.2 a	22.8 a	118.3 a
15 d at 1 °C	36.0 a	18.3 a	132.2 a	51.2 a	22.3 b	118.0 a
<i>Irradiation^x</i>						
Control	36.6	20.6	129.3	51.6	23.5	117.6
200 Gy	36.3	20.6	131.1	51.1	22.9	117.9
400 Gy	35.7	19.3	130.1	50.0	22.5	119.3
600 Gy	35.4	18.6	131.4	50.3	22.3	118.9
800 Gy	35.1	18.1	131.1	49.9	22.1	119.1
1000 Gy	35.9	19.1	129.9	50.4	22.4	118.1
<i>P</i> value	0.08	0.02	0.65	0.09	0.02	0.40
<i>Fumigation^x</i>						
Control	37.6	19.2	127.4	52.2	22.9	116.9
16 g·m ⁻³	40.2	24.6	124.3	53.7	24.3	115.7
32 g·m ⁻³	39.4	22.8	124.9	52.8	23.5	115.7
64 g·m ⁻³	41.2	25.5	122.4	53.5	24.5	114.5
<i>P</i> value	0.15	0.23	0.09	0.47	0.31	0.04

^zFor L* 100 is white and 0 is black; for C* 100 is most intense and 0 is least intense; and for h° 0° is red-purple, 90° is yellow, 180° is bluish-green, and 270° is blue.

^bMeans of 250 leaflets. Mean separation at *P* ≤ 0.05 based on analysis of variance and Dunnett's one-tailed *t* test in SAS.

^xMeans of 250 leaflets (irradiation) or 150 leaflets (fumigation). Regression analysis in SAS with treatment probabilities. Measurements of treated and untreated leaves made after 3 d at 7 °C.

with the level of irradiation appeared to be developing (*P* = 0.124); whereas untreated leaves had a cumulative weight loss of 4.40%, leaves treated at 1000 Gy lost only 3.88%.

Irradiation significantly affected the color of both the upper and lower leaf surfaces. Three days of storage at 7 °C lightened control leaves, but increased color intensity relative to freshly harvested ones (*P* ≤ 0.05; Table 1). With increasing irradiation, these effects were reversed; the L* of upper and lower surfaces was reduced, and color intensity, C*, decreased significantly. Hue was unaffected by irradiation. Subsequent storage at room temperature continued to lighten control leaves, but relationships between color and irradiation generally became stronger (data not shown). At 24 °C, surfaces of irradiated leaves continued to remain darker (*P* ≤ 0.01), and color intensity continued to decrease as the dosage of gamma irradiation increased (*P* ≤ 0.05). Although not significant at *P* ≤ 0.05, hue angle, h°, of irradiated leaves appeared to drop more slowly during ambient storage than that of untreated leaves, suggesting a reduced rate of yellowing of leaves that increased after harvest. Notwithstanding the statistics, leaves treated with as much as 1000 Gy could not be visually differentiated from untreated leaves. There was no injury associated with irradiation and no increased susceptibility to decay within 6 d of treatment (data not shown).

Methyl bromide fumigation. Weight loss of curry leaves was not affected by MB fumigation and averaged 2.4% and 5.0% after storage at 7 °C and subsequent ambient storage, respectively. At harvest, these leaves had L*, C*, and h° values of 35.28, 18.27, and 134.39 (top) and 51.96, 22.90, and 118.61 (bottom). Regression analysis did not indicate

a relationship between level of fumigation and L* or C*, and fumigated leaves were not significantly different from untreated leaves in these qualities after storage at 7 °C (Table 1). Both leaf surfaces, however, lost green hue relative to the amount of MB applied.

Injury was not apparent after fumigated leaves were held at 7 °C for 3 d. Decay, not present immediately after storage at 7 °C, was significant after three subsequent days at room temperature. On leaves not fumigated, 2.4% of the leaf surface had signs of mildew; this increased to 5.1%, 48.4%, and 87.2% as MB treatment increased from 16 to 32 and 64 g·m⁻³, respectively. Regression analysis suggested that this decay was related to fumigation (*P* = 0.03). The heavy decay concealed any injury that might have been apparent at this stage of storage and prevented another color evaluation.

Discussion

Quarantine treatments for eradication of pests from food commodities prior to export include alternatives such as heat, fumigation, cold, and irradiation (Sharp and Hallman, 1994). Heat, applied as immersion in water or in moist or dry air above 45 °C, was not considered for treatment of leaves of *M. koenigii*, since wetting could exacerbate decay, and treatment with dry heat would be cost prohibitive. Fumigation with MB appears to damage these leaves and make them susceptible to decay, although it is used successfully on many commodities, including stonefruits, nuts, and dried spices (Yokoyama, 1994). However, curry leaves tolerate standard cold treatment at 1 °C for quarantine eradication of insect pests as well as irradiation treatment to the maximum level of 1000 Gy approved for

food commodities. Any change of color was not visually apparent, and susceptibility to leaf injury or disease was not increased. Other characteristics, such as taste and aroma that are important qualities for spices, could not be evaluated since experienced consumers of this crop were not locally available.

Acceptance of any particular treatment must await mortality studies on specific insects. Of special interest currently are tests under way with the Asian citrus psyllid, which appears to tolerate irradiation to the limits available for treatment (Walter Gould, USDA, Agr. Research Service, personal communication). Although irradiation may interfere with feeding behavior and thereby reduce the threat of transmission of the citrus greening bacterium, this threat can only be totally eliminated when the insects are killed. In general, adult insects are not killed by irradiation up to 1000 Gy (Burditt, 1994).

As consumer confidence in gamma irradiation increases, this technique is being used on an increasing number of commodities for sterilization of insects at the egg and larval stages. Lychees, papayas (*Carica papaya* L.), and carambolas grown in Hawaii may be irradiated to ensure safe shipment to states on the U.S. mainland where Hawaiian fruit fly species are quarantined (Strating, 1996), and, from a general ruling on irradiation (Young and Bowen, 1986), Florida guavas (*Psidium guajava* L.) can be treated against Caribbean fruit flies prior to shipment to California and Texas. Other hosts of these and different quarantined pests have been evaluated for treatment by gamma irradiation (Burditt, 1994), but no additional protocols have yet been approved. Little information is available, however, on the effects of gamma irradiation on leafy material. Lychee leaves tolerate dosages at least to 300 Gy (McGuire, 1997), and leaves of *Leucospermum cordifolium* Salisb. ex Knight were unaffected at a similar dosage (Haasbroek et al., 1973). Leaves of *Protea compacta* R. Br. (Haasbroek et al., 1973) and chrysanthemum (*Dendranthema × grandiflora* Kitam.) (Hayashi and Todoriki, 1996), however, were blackened at 300 Gy and yellowed at 750 Gy, respectively.

Insect resistance to gamma irradiation plus its greater cost suggest that cold treatment, which is available in local packinghouses, may be preferred by growers. The results of these trials suggest that the 15-d treatment may

even be extended if killing the psyllid on curry leaves requires more time. Unfortunately, there are virtually no data on the response of surface pests to cold treatment (Mangan and Hallman, 1998). Many tropical commodities, however, can tolerate refrigeration at temperatures required for quarantine control of insect pests. Subtropical grapefruit (*Citrus paradisi* Macf.) may be shipped from Florida to Japan in containers maintained at 1.1 °C for 21 d to kill larvae of the Caribbean fruit fly (Gould, 1994). Lychees (but not longans, *Dimocarpus longan* Lour.) also tolerate cold treatment (McGuire, 1997, 1998), as do canistel (*Pouteria campechiana* Baehni) and white sapote (*Casimiroa edulis* Llave & Lex.) (Hallman and Chalot, 1993), and a treatment for carambolas was previously mentioned.

Changing demographics nationwide is fueling a diversification of agriculture in Florida and elsewhere that expands opportunities for local farmers. The cultivation of the curry leaf tree is not a large industry in Florida, but it can provide growers in the southern part of the state with an alternative crop during the winter months when fruit trees are not bearing. The continual arrival of new exotic pests by natural spread or through illegal commerce, however, is a threat on a national scale. The development of quarantine treatments, such as will result from this type of work with curry leaves, should make it more difficult for pests to establish themselves in new regions of the country.

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