

Combined postharvest X-ray and cold quarantine treatments against the Mediterranean fruit fly in ‘Clemenules’ mandarins

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Abstract

In the present work, survival of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) on artificially infested ‘Clemenules’ clementine mandarins (*Citrus reticulata* Blanco) was assessed on fruit subjected to integrated quarantine treatments consisting of irradiation with X-rays at doses of 0 (control), 30, 54, and 164 Gy followed by exposure to 1°C for 0 (control), 3, 6, 9, or 12 days. Additionally, physico-chemical (rind color, firmness, and physiological disorders, soluble solids concentration, titratable acidity, maturity index, juice yield, and ethanol and acetaldehyde content) and sensory (sweetness, acidity, sensory maturity index, off-flavors, and mandarin-like flavor) fruit quality of ‘Clemenules’ clementines were assessed on X-irradiated fruit exposed to 1°C for 0 (control), 6, or 12 days. Complete insect mortality with no negative effects on fruit quality after 7 days at 20°C of shelf life was obtained on clementines firstly X-irradiated at 30 Gy and subsequently exposed to 1°C for 2 days. This combination of treatments considerably reduced quarantine time if compared to standard cold quarantine treatments (1.1-2.2°C for 14-18 days) and therefore showed promise as a potential commercial treatment for Spanish citrus exports.

Additional key words: *Ceratitis capitata*, citrus disinfection, clementines, fruit quality, integrated quarantine treatments, sensory analysis, X-irradiation.

Resumen

Combinación en poscosecha de rayos X y frío para el tratamiento cuarentenario de mandarinas ‘Clemenules’ contra la mosca mediterránea de la fruta

En este trabajo se evaluó la supervivencia de la mosca mediterránea de la fruta *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) en mandarinas clementinas ‘Clemenules’ (*Citrus reticulata* Blanco) infestadas artificialmente y sometidas a la combinación de tratamientos ionizantes con rayos X a dosis bajas (0, 30, 54 y 164 Gy) con exposiciones a frío de corta duración (1°C durante 0, 2, 4, 6, 8, 10 ó 12 días). Adicionalmente, se determinó la calidad físico-química (color, firmeza y alteraciones fisiológicas de la corteza, concentración de sólidos solubles, acidez total, índice de madurez, rendimiento en zumo y contenidos de etanol y acetaldehído) y sensorial [dulzor, acidez, índice de madurez sensorial, malos sabores y ‘flavor’ (sabor + aroma)] de clementinas ‘Clemenules’ tratadas con rayos X y expuestas a 1°C durante 0 (control), 6 ó 12 días. Con frutos irradiados a 30 Gy y expuestos a 1°C durante 2 días se obtuvo una mortalidad completa de *C. capitata* sin efectos negativos en la calidad del fruto medida después de un período de simulación de la comercialización de 7 días a 20°C. Por tanto, esta combinación de tratamientos redujo sensiblemente los tiempos de cuarentena necesarios con los tratamientos de frío estándar (1,1-2,2°C durante 14-18 días) y mostró buen potencial como tratamiento comercial para los cítricos españoles de exportación.

Palabras clave adicionales: análisis sensorial, calidad de fruto, *Ceratitis capitata*, clementinas, desinsectación de cítricos, irradiación, tratamientos de cuarentena integrados.

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Received: 10-04-07; Accepted: 03-10-07.

Introduction

Worldwide plantings and consumption of clementine mandarins have been steadily increasing during the last years. The Spanish cultivar 'Clemenules' (syns.: 'Clementina de Nules', 'Nules') is the leading clementine produced around the world. Spain is the world's largest exporter of fresh citrus fruit, including clementine mandarins (FAO, 2004). Export shipments to overseas markets such as the USA and Japan are arising because, in comparison to other mandarin-type citrus fruit, clementines are of high organoleptic quality, seedless, and very easy to peel. The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the most damaging fruit pests worldwide and may be a major pest of citrus (White and Elson-Harris, 1992; EPPO, 2007). Currently, many countries maintain strict quarantine measures against this pest (EPPO, 2007). Citrus exports from Spain to *C. capitata*-free countries are subjected to a mandatory cold-based quarantine treatment. In the case of the USA, the Department of Agriculture established a minimum exposure during overseas transit of 14-18 days at 1.1-2.2°C (USDA, 2002b). These periods of cold quarantine were established after a re-evaluation of the scientific basis for previous regulatory treatment schedules that was conducted as a consequence of interceptions, in recent years, of live *C. capitata* larvae in fruit that had been cold-treated during transit (USDA, 2002a, 2004). Extensive research is currently focused on the development of alternative or complementary quarantine treatments, especially for cold sensitive commodities such as a variety of citrus cultivars. Irradiation treatments are known to be effective against fruit flies and other pests of quarantine importance (Burditt, 1994; Hallman, 1999) and they could be one of such viable alternatives for Spanish citrus exports.

Ionizing radiation sources for food treatment include radioactive (^{60}Co or ^{137}Cs , γ -irradiation) and machine sources (electron beams and X-rays). An advantage of machine sources is that the use of radioactive isotopes is not required. When treating fresh fruit, however, high energy electrons are difficult to use due to their poor power of penetration into produce (McLaughlin, 1999). This problem could be overcome with the use of X-rays. When high-energy electrons are directed toward a metallic target, it produces X-rays, which have a much higher penetration power. Therefore, horticultural products of large size and also pallet-

loads of produce can be treated provided that they pass the irradiation source on a conveyor line (Hallman, 1999). The US Food and Drug Administration approved the use of X-rays from a source with beam energy of ≤ 5 MeV for food irradiation at a dose of $\leq 1,000$ Gy (US FDA, 1986). This rule has been amended to include an energy level of ≤ 7.5 MeV for X-rays generated from machine sources using tantalum or gold as the target material (US FDA, 2004). The USDA established a minimum absorbed dose of 225 Gy for quarantine purposes against *C. capitata* (USDA, 2002c). Nevertheless, this dose was lowered based on recent research (Hallman and Loaharanu, 2002; Follett and Armstrong, 2004), and a new rule established a generic treatment dose of 100 Gy against fruit flies (USDA, 2006). This target dose, however, can vary by as much as 2 to 3-fold when treating fresh fruit on a commercial scale (Hallman, 1999; Boylston *et al.*, 2002) and this possible variation should be taken into account to assess the effects of irradiation on fruit quality. In general, the efficacy of ionizing radiation treatments and their effects on the quality of citrus fruit is affected by factors related to the fruit itself (e.g. cultivar, physical and physiological condition), the irradiation treatment (e.g. source, dose), and the postharvest fruit handling (e.g. postharvest treatments, storage conditions). As a function of these factors, no substantial effects or both beneficial (extension of shelf-life) and detrimental (rind injuries) effects have been reported for citrus fruit (Maxie *et al.*, 1969; Miller *et al.*, 2000; Mahrouz *et al.*, 2002, 2004; Patil, 2004; Alonso *et al.*, 2007). According to Follett and Neven (2006), since the effects of insecticidal treatments on treated produce can considerably differ depending on species and cultivar, varietal testing is essential to establish the tolerance of fruits and vegetables to different quarantine treatments. Provided that both the efficacy against the fruit fly and fruit quality are not negatively affected, the integration of the use of low doses of X-rays with reduced periods of cold quarantine may be a feasible approach for Spanish clementines to shorten the required total quarantine time, reduce potential cold damage and treatment costs, and facilitate commercial trade.

Therefore, objectives of the present work were to: 1) determine the effects of the integration of X-irradiation and reduced periods of cold exposure (1°C) on the survival of *C. capitata* on artificially infested 'Clemenules' clementine mandarins and 2) assess the effects of such sequence of quarantine treatments on the physico-chemical and sensory quality of the fruit.

Material and Methods

Fruit

Clementine mandarins (*Citrus reticulata* Blanco) cv. Clemenules from orchards in the Valencia area were hand-harvested at commercial maturity and transferred to the IVIA postharvest facilities where they were selected, randomized, washed with tap water, dipped in a mixed fungicide solution of imazalil (2,500 ppm) and guazatine (800 ppm) for 1.5 min, and waxed with a commercial wax containing 10% of total solids and 0.5% of thiabendazole. Subsequently, the fruit were separated into four homogeneous groups of 100 fruit each plus four groups of 450 fruit each. The former 400 fruits were artificially infested with *C. capitata* and used to assess the effects of the experimental quarantine treatments on insect survival. The remaining 1,800 fruit were placed in lidded commercial 40×2×27 cm cardboard boxes (150 fruit per box) and used to assess the effects on fruit quality. About 150 additional clementines were used to determine fruit quality at harvest (initial quality).

Rearing of *Ceratitis capitata*

Insects used in this study originated from a laboratory colony maintained at the IVIA. This colony was established in 2001 and has been periodically supplemented with the introduction of wild flies from naturally occurring infested fruit during summer and fall. Adults and immature stages of *C. capitata* were reared in controlled environmental conditions (25±1°C, 75±5% relative humidity (RH), and artificial illumination) as described in detail by Alonso *et al.* (2005). Third instar larvae, the most robust stage of this fly against insecticidal treatments including irradiation (Balock *et al.*, 1963) were used in all assays.

Fruit infestation

Mandarins were artificially infested with *C. capitata* larvae by puncturing and removing with a cork borer a plug 10 mm in diameter and 20 mm in deep from each fruit and introducing 10 third instar larvae into each fruit hole (Alonso *et al.*, 2005). The plug of rind and pulp previously removed was then returned to the fruit and sealed with warm paraffin applied with a soft paintbrush.

Infested fruit were placed in four separate lidded 40×29×27 cm cardboard boxes (100 fruit per box).

X-ray irradiation

The boxes containing either infested or non-infested mandarins were transported in a conditioned truck to the irradiation plant (Beta Gamma Service, BGM, Bruchsal, Germany). During transportation, the fruit were kept at 20±3°C. About 36 h later, the fruit were exposed to X-rays from a source with beam energy of 0.8 MeV and a conveyor speed of 5 m min⁻¹. The following theoretical doses were selected: 0 (control), 25, 50, and 150 Gy. Each dose was applied to one box of *C. capitata*-infested fruit and three boxes of non-infested fruit. Actual doses were determined by placing 2-cm² radiochromic dosimetry films (Gafchromic® HD-810, International Specialty Products, Wayne, NJ, USA) at three different heights within three different boxes. Readings (nine per dose) were made with a spectrophotometer at 560 nm and mean and standard error values were 30±1, 54±1, and 164±4 Gy for the respective theoretical doses. Control fruit were not irradiated; they were kept at 20°C until the application of the cold quarantine treatments.

Cold quarantine treatments

C. capitata-infested and irradiated mandarins were divided into groups and held at 1±0.5°C for 0 (control), 2, 4, 6, 8, 10, or 12 days in a 40-m³ research cold storage room. For each irradiation dose and cold quarantine time, 10 to 14 mandarins were used. To assess fruit quality, non-infested and irradiated mandarins were held at the same temperature for 0 (control), 6, or 12 days. For each irradiation dose, each cold quarantine treatment was applied to 150 mandarins.

Assessment of survival of *Ceratitis capitata*

On completion of each combination of quarantine treatments, infested and treated fruit were individually kept at 25±1°C and 80±5% RH in 1-L cloth-covered plastic boxes where alive larvae were freely allowed to emerge from the fruit. After a period of 20 to 25 days in these environmental conditions, the number of pupae and adults of *C. capitata* in the box was counted

and the fruit was opened to account for non-emerged dead larvae. From these data, pupariation and survival percentages referred to the initial number of inoculated larvae were obtained. Each fruit was considered as a replicate.

Fruit quality assessment

External and internal physico-chemical quality and sensory quality of mandarins were determined at harvest and after the corresponding combination of quarantine treatments plus a shelf life period of 7 days at 20°C to simulate prompt fruit commercialization. Quality attributes were determined as follows:

Rind color

Rind color was measured as Hunter parameters (L, a, b) with a colorimeter (Minolta, Model CR-300). A specific color index (CI) for citrus was calculated as $CI = 1,000a/L * b$ (Jiménez-Cuesta *et al.*, 1981). For each treatment, three measurements along the equatorial area of 60 fruit were performed.

Fruit firmness

Firmness of 60 fruit per treatment was determined at the end of shelf life using an Instron Universal Testing Machine (Instron Corp., Model 4301). Each fruit was compressed between two flat surfaces closing together at the rate of 5 mm min⁻¹. The machine gave the deformation (mm) after application of a load of 10 N to the equatorial region of the fruit. Results were expressed as percentage of deformation related to initial diameter.

Physiological disorders

External rind injuries potentially produced by X-irradiation or low temperature (basically rind browning or pitting) were assessed by the naked eye at the end of the shelf life period on 30 randomized fruit for each treatment. Each fruit was classified into one of the four following categories according to rind damage: 0 = none, 1 = light, 2 = moderate, and 3 = severe. Light was considered when less than 10% of fruit surface was affected and severe when this percentage was above 20%. Results on incidence and severity of disorders

were converted to a ponderate average value per treatment (0-3 scale).

Maturity index

The juice from nine replicates of 10 fruit each was used to determine a maturity index (MI). Soluble solids concentration (SSC) was measured with a digital refractometer (Atago, Model PR1) and expressed as percentage (°Brix). Titratable acidity (TA) was determined from a 5-mL aliquot by titration with 0.1 N NaOH and phenolphthalein indicator and given as g of citric acid per 100 mL of juice. MI was calculated as SSC/TA ratio.

Juice yield

For each quarantine treatment, the juice from 90 previously weighed clementines was obtained with a rotatory citrus squeezer and filtered through a 0.8-mm diameter sieve. Juice yield was expressed as percentage of juice volume (mL) per fruit weight (g).

Juice volatiles

Ethanol and acetaldehyde concentrations in the juice were determined by headspace gas chromatography according to the method described by Ke and Kader (1990). For each quarantine treatment, nine replicates of 10 fruit each were analyzed. Five mL of juice was transferred to 10-mL vials with crimp-top caps and TFE/silicone septum seals and kept at -18°C. The vials were equilibrated in a water bath at 20°C for 1 h followed by 10 min at 30°C. Then, 1-mL sample from the headspace was withdrawn from each vial and injected in a gas chromatograph (Perkin Elmer, Model 2000) with a flame ionization detector and a 1.2 m × 0.32 cm Porapack QS 80/100 stainless steel column. The injector was set at 175°C, the column at 150°C, the detector at 200°C, and the carrier gas (He) at 62.7 kPa. Volatiles were identified and quantified by comparison of retention times with standards. Results were expressed as mg per 100 mL of juice.

Sensory quality

Twenty mandarins per each irradiation treatment were sampled in a sensory analysis session for each of

the three cold quarantine periods. Each mandarin was peeled and its segments separated. Three random segments were placed on white pots identified by a random three-digit code. Two different tests were developed: a descriptive analysis to evaluate the sensory characteristics of the fruit and a difference test to determine whether a sensory difference existed between two samples with no specification of which sensory attribute(s) were causing the difference. All judges were 23-50 year-old volunteers selected among the IVIA staff. All evaluations were conducted in individual booths under white illumination at room temperature in a EU homologated sensory room. Mineral water was used as palate cleanser between samples (AENOR, 1997).

For the descriptive analysis, eight trained panelists (three men and five women who had attended twenty three 30-min sessions to discriminate and describe different attributes of citrus fruit) were asked to rate sweetness, acidity, and sensory MI according to a 15-cm line scale. Mandarin-like flavor was evaluated using a 9-point scale, where points 1-3, 4-6, and 7-9 designated bad quality, acceptable quality, and high quality, respectively. Off-flavors were evaluated using a 6-point scale where points 0, 1, 2, 3, 4, and 5 designated none, slight, slight-moderate, moderate, moderate-strong, and strong, respectively. The order of presentation of the pots was randomized for each judge in every session; six pots were evaluated, two replicates for each X-ray treatment.

A triangle test with semitrained panelists (Meilgaard *et al.*, 1999) was chosen as difference test. The sensory panel consisted of three men and seven women, all of them habitual consumers of mandarins. Six triads with samples from different irradiation treatments were evaluated at each sensory session. Samples were swallowed and re-tasting was permitted. The presentation order of treatment comparison was counter-balanced across panelists and sample presentation was randomized within triads.

Statistical analysis

Analyses of variance (ANOVA) were performed for both pupariation and adult emergence percentages as well as for each quality parameter using Statgraphics Plus 4.1 (Manugistics Inc.). Data from countings were arcsine-transformed prior to analysis. Where appropriate, means were separated by Fisher's Protected LSD test

($P=0.05$). For the sensory triangle test, statistical analysis was based on the number of correct answers and it was calculated using specific tables (AENOR, 1997; $P=0.05$).

Results

Survival of *Ceratitis capitata*

The percentage of pupariation of third instar larvae of *C. capitata* artificially infested in 'Clemenules' mandarins significantly decreased as both X-ray dose and time of exposure to 1°C increased (Fig. 1; $F=44.95$; $df=3, 273$; $P<0.0001$; and $F=52.87$; $df=6, 273$; $P<0.0001$, respectively). Furthermore, the interaction between these two factors was significant ($F=2.0$; $df=18, 273$; $P=0.0102$). A complete inhibition of pupariation was only achieved on insects treated at 164 Gy and exposed to 1°C for 12 days. On the contrary, complete inhibition of adult emergence occurred after X-irradiation at the lowest dose of 30 Gy followed by the shortest cold exposure period (2 days) (Fig. 2). Adult emergence also significantly decreased as both X-ray dose and time of exposure to 1°C increased ($F=236.17$; $df=3, 270$; $P<0.0001$; and $F=16.87$; $df=6, 270$; $P<0.0001$; respectively). In this case the interaction was also significant ($F=15.74$; $df=18, 270$; $P<0.0001$). When cold quarantine was applied to non-irradiated infested mandarins, the longest period of 12 days at 1°C was needed to achieve complete mortality of *C. capitata*.

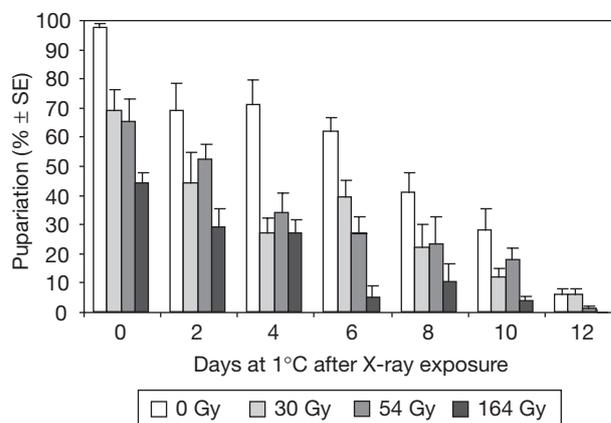


Figure 1. Pupariation of *Ceratitis capitata* third instar larvae artificially infested in 'Clemenules' mandarins, irradiated with X-rays at 0, 30, 54, or 164 Gy, exposed to 1°C for 0, 2, 4, 6, 8, 10, or 12 days and held at 25°C for 20-25 days.

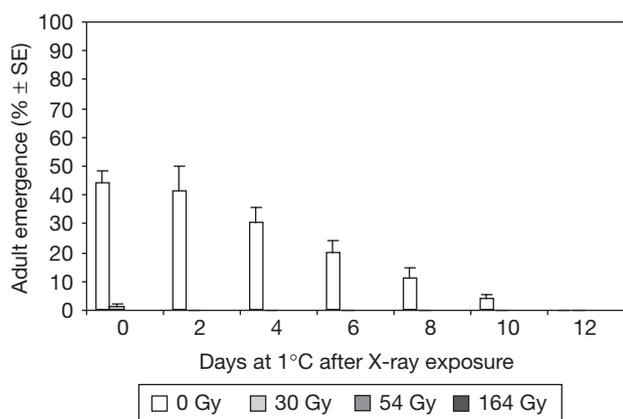


Figure 2. Adult emergence from *Ceratitis capitata* third instar larvae artificially infested in 'Clemenules' mandarins, irradiated with X-rays at 0, 30, 54, or 164 Gy, exposed to 1°C for 0, 2, 4, 6, 8, 10, or 12 days and held at 25°C for 20-25 days.

Fruit quality

Physico-chemical quality

External physico-chemical quality attributes of 'Clemenules' mandarins were minimally affected by the integrated quarantine treatments (Table 1). Irrespective of the X-ray dose, rind CI and percentage of rind deformation were slightly higher on cold-treated than on control clementines. Rind CI was consistently lower at harvest (more greenish rind color) than at the end of shelf life. After 12 days of cold exposure, CI

values for non-irradiated and 30 Gy-irradiated fruit were significantly higher than for 54 or 164 Gy-irradiated fruit. Irradiated mandarins were in general less firm (higher values of rind deformation) than non-irradiated ones. No external physiological disorders were observed, thus none of the quarantine treatments caused any rind damage.

Similarly, internal physico-chemical quality attributes of clementines were only slightly affected by the different combinations of quarantine treatments. Although significant in some cases, the differences were minimal and had no practical impact (Table 2). While TA and juice yield were lower, SSC and MI were higher after shelf life than at harvest. In general, differences in TA, SSC, MI, and juice yield between non-irradiated and irradiated fruit were not significant. As expected, acetaldehyde and particularly ethanol content in the juice were higher after cold exposure. Ethanol content increased from 6.37 mg/100 mL of juice at harvest to 45.83 and 55.41 mg/100 mL of juice in fruit treated at 1°C for 12 days that had been previously irradiated at 54 and 164 Gy, respectively. In general, the content of both volatiles were significantly higher on X-irradiated mandarins.

Sensory quality

Irrespective of the period of cold quarantine, trained panelists did not find significant differences in sweetness,

Table 1. External physico-chemical quality attributes of 'Clemenules' mandarins irradiated with X-rays at 0, 30, 54, or 164 Gy, exposed to 1°C for 0, 6, or 12 days, and kept at 20°C for 7 days of shelf life

Cold quarantine period (days) ¹	X-ray dose (Gy)	Rind color index (CI) ²	Rind firmness (% deformation)	Physiological disorders (0-3 scale)
Initial (at harvest)		4.24	6.94	0
0 (control)	0 (control)	6.61 a	6.01 a	0
	30	6.20 a	6.27 a	0
	54	6.20 a	6.01 a	0
	164	6.17 b	6.73 b	0
6	0 (control)	8.35 b	8.79 a	0
	30	7.49 a	9.35 b	0
	54	8.24 b	9.35 b	0
	164	7.76 ab	9.52 b	0
12	0 (control)	8.89 b	9.30 a	0
	30	9.13 b	10.50 b	0
	54	7.99 a	9.49 a	0
	164	7.89 a	9.43 a	0

¹ For each cold quarantine period, means within columns followed by unlike letters are different according to Fisher's Protected LSD test ($P=0.05$). ² CI = 1,000a/L*b; Hunter parameters.

Table 2. Internal physico-chemical quality attributes of ‘Clemenules’ mandarins irradiated with X-rays at 0, 30, 54, or 164 Gy, exposed to 1°C for 0, 6, or 12 days, and kept at 20°C for 7 days of shelf life

Cold quarantine period (days) ¹	X-ray dose (Gy)	TA ² (%)	SSC ³ (%)	Maturity index (MI) ⁴	Juice yield (%)	Ethanol (mg/100 mL)	Acetaldehyde (mg/100 mL)
Initial (at harvest)		1.30	9.69	7.45	48.30	6.37	0.50
0 (control)	0 (control)	1.25 a	9.93 a	7.94 a	37.93 b	20.02 b	0.67 b
	30	1.20 a	9.82 a	8.18 ab	36.30 a	16.38 a	0.59 a
	54	1.20 a	10.07 a	8.38 b	36.26 a	24.05 c	0.71 bc
	164	1.19 a	9.82 a	8.25 b	35.55 a	28.18 d	0.74 c
6	0 (control)	0.98 a	9.96 a	10.22 a	29.20 a	50.18 c	1.27 b
	30	0.99 a	10.04 a	10.11 a	30.92 a	39.91 b	1.04 a
	54	1.04 a	10.24 ab	9.95 a	32.98 a	32.99 a	1.03 a
	164	1.02 a	10.45 b	10.27 a	31.49 a	53.30 c	1.59 c
12	0 (control)	0.95 a	9.95 a	10.52 b	29.16 a	25.04 a	0.84 a
	30	0.99 ab	10.45 ab	10.54 b	31.22 a	27.76 a	0.83 a
	54	1.00 ab	10.24 b	10.28 b	31.35 a	45.83 b	1.48 c
	164	1.06 b	10.13 c	9.61 a	31.52 a	55.41 c	1.20 b

¹ For each cold quarantine period, means within columns followed by unlike letters are different according to Fisher’s Protected LSD test ($P=0.05$). ² TA: titratable acidity. ³ SSC: soluble solids concentration. ⁴ MI: SSC/TA

acidity, and sensory MI between non-irradiated and irradiated clementines. The judges detected slight to slight-moderate off-flavors on fruit that had been X-irradiated at 164 Gy and exposed to 1°C for 0 or 6 days. Although fruit from all treatments were rated at least as acceptable for mandarin-like flavor, non cold-

treated mandarins were scored higher than the rest of fruit (Table 3). In the triangle test, the untrained panel only found significant differences between control (non-irradiated) fruit and fruit X-irradiated at 54 or 164 Gy when mandarins had been exposed to 1°C for 6 days (data not shown).

Table 3. Sensory quality attributes of ‘Clemenules’ mandarins irradiated with X-rays at 0, 30, 54, or 164 Gy, exposed to 1°C for 0, 6, or 12 days, and kept at 20°C for 7 days of shelf life

Cold quarantine period (days) ¹	X-ray dose (Gy)	Sweetness (15 cm scale)	Acidity (15 cm scale)	Sensory MI (15 cm scale)	Off-flavors (0-5 scale)	Mandarin-like flavor (1-9 scale)
Initial (at harvest)		7.90	7.75	7.45	1.20	7.00
0 (control)	0 (control)	8.83 a	7.40 a	8.15 a	1.63 a	6.18 a
	30	8.38 a	7.58 a	7.75 a	1.33 a	6.38 a
	54	8.39 a	7.34 a	7.80 a	1.67 a	6.13 a
	164	8.04 a	7.46 a	7.76 a	2.75 b	5.11 a
6	0 (control)	8.06 a	5.24 a	8.25 a	2.45 a	4.25 a
	30	8.53 a	6.58 a	8.20 a	2.08 a	5.66 b
	54	8.62 a	6.90 a	8.05 a	1.83 a	5.66 b
	164	8.92 a	5.53 a	8.38 a	3.58 b	3.58 a
12	0 (control)	7.86 a	6.10 a	7.43 a	2.41 a	4.37 a
	30	8.87 a	6.86 a	7.99 a	1.58 a	5.83 a
	54	8.15 a	7.29 a	7.42 a	2.12 a	4.62 a
	164	8.74 a	7.05 a	7.86 a	2.16 a	5.29 a

¹ For each cold quarantine period, means within columns followed by unlike letters are different according to Fisher’s Protected LSD test ($P=0.05$).

Discussion

Synergistic effects between X-ray ionizing radiation and low temperature exposure on the mortality of *C. capitata* in infested 'Clemenules' mandarins were observed in these experiments. Both pupariation and adult emergence significantly decreased as both X-ray dose and time of exposure to 1°C increased. Complete inhibition of adult emergence of *C. capitata* was achieved with the combination of treatments at X-ray doses lower than the generic irradiation dose of 100 Gy recently established by the US legislation as the minimum dose needed for effective quarantine treatment against *C. capitata* (USDA, 2006). Furthermore, the integration of these two complementary treatments resulted in a reduction from 14 to 2 days of the time of exposure to low temperature required for satisfactory control of the Mediterranean fruit fly (USDA, 2002a, 2004). Therefore, the commercial implementation of these combined treatments would largely improve the current standard quarantine protocols by reducing the duration of the treatments and allowing the treatment of citrus exports that are especially susceptible to injuries caused by either ionizing radiation or cold exposure. Similar results were obtained by Von Windeguth and Gould (1990) for the Caribbean fruit fly, *Anastrepha suspensa* (Loew.), in grapefruit. These authors proposed a combination of 50 Gy and 5 days at 1.1°C as an acceptable method of quarantine for this citrus species.

Although the differences had no commercial impact, rind orange color of irradiated 'Clemenules' mandarins was slightly less intense than that of non-irradiated control fruit. We also observed this «loss» of color in 'Clemenules' clementines X-irradiated at higher doses of 195 and 395 Gy (Alonso *et al.*, 2007) and in 'Nova' mandarins irradiated with high-energy electrons at 500 and 1,000 Gy (Alonso *et al.*, 2004). The same trend was observed after exposure to γ -rays by Mahrouz *et al.* (2002) on 'Nour' mandarins and Maxie *et al.* (1969) on 'Washington Navel' and 'Valencia' oranges. On the other hand, rind CI after shelf life was higher than at harvest and also higher on clementines exposed to 6 or 12 days at 1°C than on non cold-treated fruit. This was not an unexpected result and it is in agreement with results of other research in which citrus fruit had been exposed to low temperature (Martínez-Jávega *et al.*, 2004; Alonso *et al.*, 2005, 2007; Barry and van Wyk, 2006).

A decrease in fruit firmness during storage following irradiation has been extensively described for different

citrus species including mandarins (Abdellaoui *et al.*, 1995; Miller *et al.*, 2000; Alonso *et al.*, 2002, 2004, 2007). Since this decrease is dose-dependent and fruit firmness also diminishes during cold storage and commercialization, it can be recommended not to store for long periods previously irradiated citrus fruit. However, the present and previous research (Alonso *et al.*, 2007) confirm that rind firmness of 'Clemenules' clementines is not adversely affected by exposure to X-rays at doses as high as 395 Gy.

In contrast to previous work in which some rind browning on waxed 'Clemenules' mandarins occurred after X-ray treatment at 195 or 395 Gy (Alonso *et al.*, 2007), no external physiological disorders were observed in these tests.

In general, the cultivar is a major factor influencing the susceptibility of citrus fruit to postharvest physiological disorders, including those caused by low temperatures and ionizing radiation (Lafuente and Zacarías, 2006). In a study on the effects of γ -rays at doses ranging from 150 to 450 Gy on the quality attributes of selected mandarin cultivars, Miller *et al.* (2000) found a wide range of tolerance to irradiation damage (from 1.7 to 100% peel pitting in 'Minneola' and 'Sunburst' mandarins, respectively). Ladaniya *et al.* (2003) observed no rind disorders in 'Nagpur' mandarins after γ -irradiation at 1,500 Gy. In contrast, unacceptable damage on 'Fortune' mandarins was noticed after electron irradiation at 1,000 Gy (Alonso *et al.*, 2002).

In this and other studies (Miller *et al.*, 2000; Boylston *et al.*, 2002; Mahrouz *et al.*, 2002; Alonso *et al.*, 2004, 2007), exposure to low temperature had a greater impact than irradiation on internal maturity and juice yield of citrus fruit. As expected, juice TA and SSC were lower and higher, respectively, in quarantine treated mandarins than at harvest. MI and juice yield of mandarins increased and decreased, respectively, after the application of the combined quarantine treatments, especially on cold-treated fruit. Nevertheless, X-irradiation did not consistently affect these quality attributes. In general, X-ray treated 'Clemenules' showed higher concentration of both ethanol and acetaldehyde than control fruit after cold exposure, especially when treated at the highest dose of 164 Gy. Although some significant differences in the incidence of off-flavors were detected by the judges in the sensory evaluation, the biosynthesis of these volatile compounds was not high enough to negatively affect the flavor of the fruit. Moreover, the critical ethanol level of 200 mg/100 mL of juice, which has been associated to build-up of off-

flavors in citrus fruit (Ke and Kader, 1990; Hagenmaier, 2002), was not exceeded. These results are in agreement with those of other authors working with different mandarin cultivars (Abdellaoui *et al.*, 1995; Miller *et al.*, 2000; Mahrouz *et al.*, 2002; Ladaniya *et al.*, 2003; Alonso *et al.*, 2004, 2007). Nevertheless, volatile synthesis in response to irradiation has been found to be cultivar-dependent (Miller *et al.*, 2000) and in some cases irradiation can lead to unacceptable contents. For instance, values of ethanol in excess of 800 mg/100 mL were obtained after irradiation and cold storage of 'Fortune' mandarins with electron beams at 1,000 Gy (Alonso *et al.*, 2002).

With the exception of off-flavors, the sensory attributes of 'Clemenules' mandarins were not influenced by the combined quarantine treatments. The significant differences detected by the untrained panel in the triangle test were not related to the period of cold quarantine. O'Mahony *et al.* (1985) did not find differences between irradiated and no-irradiated 'Navel' oranges in a 3-AFC (three alternative forced choice) test when minimum differences were found in flavor by a trained panel. Therefore, even the most aggressive combination of treatments consisting of X-irradiation at 164 Gy plus exposure at 1°C for 12 days did not reduce consumer acceptance. Similar results were obtained by Miller *et al.* (2000) with 'Minneola', 'Murcott' and 'Fallglo' mandarins and Ladaniya *et al.* (2003) with 'Nagpur' mandarins irradiated with doses up to 1,500 Gy, and by Hallman and Martínez (2001) with 'Dancy' tangerines irradiated with doses from 150 to 5,000 Gy. Likewise, sensory evaluation (aroma and flavor) of 'Kau Gold' oranges was not affected by exposure to X-irradiation at 75 Gy (Boylston *et al.*, 2002). Furthermore, Mahrouz *et al.* (2002) reported a beneficial effect in sensorial and nutritive properties of 'Nour' mandarins irradiated at 300 Gy. Irradiated fruit were sweeter and had higher contents of vitamin C and total phenolic compounds.

Since X-ray irradiation at low doses followed by cold exposure for short periods had no detrimental effects on fruit quality and effectively controlled the Mediterranean fruit fly, such combined treatments could be as useful as the current standard disinfestation quarantine treatment for 'Clemenules' mandarins. Therefore, the combination of quarantine treatments consisting of X-irradiation at 30 Gy plus 2 days of fruit exposure to 1°C should be further tested at a larger scale for evaluation of its possible implementation as a commercial quarantine treatment. The integration of treatments would provide clear technological benefits

for the Spanish citrus industry because it would be a non-polluting approach that would substantially reduce quarantine time and allow the treatment of citrus cultivars susceptible to either irradiation or chilling injury while maintaining the reliability of the standard cold quarantine protocols. Provided that treatment plants become more common in the near future, this approach should not result in a too high added cost.

Acknowledgments

This work was partially funded by the Spanish INIA and the EU FEDER program (project INIA RTA03-103-C6-1). Ms. Alicia Marcilla's doctorate program was supported by the INIA. Mr. Alfonso Kurtz is gratefully acknowledged for his technical assistance.

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