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Phytosanitary irradiation of peach fruit moth (Lepidoptera: Carposinidae) in apple fruits

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HIGHLIGHTS

- Dose–response tests were conducted on eggs and all larval stages.
- Fifth instar is the most tolerant stage that could be shipped in fruits.
- None normal-looking adult emerged from 30,850 fifth instars in confirmatory tests.
- A minimum of 228 Gy is suggested for phytosanitary irradiation of peach fruit moth.

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ABSTRACT

Peach fruit moth, *Carposina sasakii* Matsumura, is a serious pest of many pome and stone fruits and presents a quarantine problem in some export markets. It is widely distributed in pome fruit production areas in China, Japan, Korea, North Korea and the Far Eastern Federal District of Russia. In this investigation, gamma radiation dose–response tests were conducted with late eggs (5-d-old) and various larval stages, followed by large-scale confirmatory tests on the most tolerant stage in fruit, the fifth instar. The dose-response tests, with the target radiation dose of 20 (late eggs), 40, 60, 80, 100, 120, 140, and 160 Gy (late fifth instars in vitro) respectively applied to all stages, showed that the tolerance to radiation increased with increasing age and developmental stage. The fifth instar (most advanced instar in fruits) was determined to be the most tolerant stage requiring an estimated minimum absorbed dose of 208.6 Gy (95% CI: 195.0, 226.5 Gy) to prevent adult emergence at 99.9968% efficacy (95% confidence level). In the confirmatory tests, irradiation was applied to 30,850 late fifth instars in apple fruits with a target dose of 200 Gy (171.6–227.8 Gy measured), but only 4 deformed adults emerged that died 2 d afterwards without laying eggs. A dose of 228 Gy may be recommended as a phytosanitary irradiation treatment under ambient atmosphere for the control of peach fruit moth on all commodities with an efficacy of 99.9902% at 95% confidence level.

1. Introduction

Peach fruit moth, *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae), is one of the most destructive borers of pome and stone fruits, including apple (*Malus* spp.), pear, peach, plum, apricot (all *Prunus* spp.), Chinese quince (*Chaenomeles sinensis*), jujube (*Zizyphus jujube*), and hawthorn (*Crataegus* spp.) in China (Huang and Wu, 1975; EPPO, 1997; Li D.X. et al., 2012). It is widely distributed in pome fruit production areas in China, Japan, both Koreas, and the Far Eastern Federal District of Russia (Hua, 1992; EPPO, 1997). Several *C. sasakii* eggs are laid on one fruit, usually near the calyx (EPPO, 1997). The newly hatched larvae crawl on the fruit surface for 10–30 min to find a proper entrance site and then bite the surface and enter into the fruits (Hou and Hua, 2004), resulting in fruit malformation, deterioration of fruit quality, and loss of marketability (Hua, 1992; EPPO, 1997). *C. sasakii* is a quarantine pest of concern to the European Union, the United States of America, Brazil, Chile, Canada, South Africa, Russia and other importing countries (EPPO, 1997). To protect fruits from infestation, fruit bagging technology is widely applied in apple, pear, and other fruits. However, this kind of technology is unable to impede the infestation entirely and has a negative impact on fruit quality (Li A.H. et al., 2012; Li D.X. et al., 2012; Shen et al., 2012). Therefore, a phytosanitary treatment often must be applied on the fruits before export.

Methyl bromide fumigation is currently the only effective measure for phytosanitary treatment of peach fruit moth in host fruits (EPPO, 1997; Liu et al., 2010). A fumigation schedule for phytosanitary treatment of *C. sasakii* infesting fresh fruits was established in Russia

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and China and accepted by most importing countries (EPPO, 1997; Li L. et al. 2012). Methyl bromide is a stratospheric ozone-depleting substance defined by the Montreal Protocol on Substances that Deplete the Ozone Layer, and reducing its use and emissions in quarantine and pre-shipment (QPS) treatment is sought and alternative quarantine treatments are needed (TEAP, Technology and Economic Assessment Panel, 2009; Hallman, 2011).

Ionizing radiation is a relatively new commercially used phytosanitary treatment that is increasing in use worldwide (Hallman, 2011). A dose of 500 Gy may prevent 100% of F_1 progeny eggs hatching from the late pupae (1–3 d before adult emergence) of *C. sasakii* (Zhang and Wang, 1983). Nevertheless, *C. sasakii* late fifth instars typically leave the fruit for pupation and the immature larvae may survive for a long period in stored fruits (EPPO, 1997); the most advanced stage found in shipped fruits is the fifth instar. To facilitate the application of phytosanitary irradiation (PI) there is a need to develop specific doses for quarantine Lepidoptera and reduction of dose levels for specific pests and commodities (Follett, 2009). An international coordinated research project under the auspices of the Joint FAO/IAEA Programme on Nuclear Techniques in Food and Agriculture was initiated in 2009 to develop generic irradiation doses for quarantine treatments of pest other than fruit flies (IAEA, 2009). Consequently, the objective of this research was to establish a PI treatment dose for peach fruit moth in host fruits. This requires conducting dose–response tests to determine the most tolerant stages infesting fruits, and treating a minimum of 30,000 late fifth instars (the most tolerant stage in fruits) with no normal-looking adult emergence in the confirmatory tests (Couey and Chew, 1986; FAO, 2003; IAEA, 2009; FAO, 2007).

2. Materials and methods

2.1. Insects rearing

The insects used in the dose–response tests were from *C. sasakii* infested apples growing in Heibei Province, China and replaced in 2009 (Liu et al., 2010). In 2011, the insects were replaced with individuals collected from hawthorn fruits growing in Taian city, Shandong Province, China. The hawthorn colony, which has the same response (mortality to adult stage) to gamma radiation as the apple colony (Zhan et al., 2014), was used for conducting the confirmatory tests. All the stages were reared at 24–26 °C, 70–90% RH with a photoperiod of 15:9 (L:D) in the Laboratory of Quarantine Treatment and Equipment, Chinese Academy of Inspection and Quarantine, Beijing, China. Paired adults (10 females:12 males) were fed a 5% honey water solution through a wick, and the females laid eggs on rough filter papers. The paper containing 5-d-old eggs (head dark stage) was wetted and stuck to the calyx end of 'Red Fuji' apples (brought form Beijing market and allowed to reach room temperature) and placed in a steel tray, which was wetted and sealed with thin plastic sheet for 3 d to maintain high relative humidity (~90% RH) to facilitate egg hatching. The late fifth instars emerging out of the infested apples were transferred to moist pine sawdust for pupation and adult emergence.

2.2. Experimental design

2.2.1. Dose–response test on late eggs

5-d-old eggs on the filter paper with 100 individuals on each were subjected to gamma radiation at the target doses of 20, 40, 60, 80, 100, 120, and 140 Gy; each dose was replicated three times.

2.2.2. Dose–response tests on larvae in apples

All five larval stages of *C. sasakii* infested in apples were irradiated simultaneously with a series of doses between 40 and 140 Gy at 20-Gy increments; each dose was replicated three times. The infested apples were prepared by injecting 5-d-old eggs into "Red Fuji" apples (~20 eggs/apple) 15, 12, 9, 6, and 3 d before irradiation.

2.2.3. Dose–response test on late fifth instars in vitro

The late fifth instars were collected within 24 h before radiation treatment; 50 insects were placed in 50-ml plastic cups (with 1/3 volume of sawdust), and irradiated at series doses between 40 and 160 Gy at 20-Gy increments. Each dose was replicated six times.

2.2.4. Confirmatory tests

To confirm the minimum dose to provide quarantine security at the 99.99% level of prevention of adult emergence (95% confidence level), a target dose of 200 Gy (based on the probit analysis) was applied to the apples infested with 15-d-old larvae (5th instars). The confirmatory test was repeated 5 times until the cumulative number of insects treated exceeded 30,000.

2.3. Irradiation treatment

2.3.1. Irradiator

All the irradiation treatments were conducted at the National Institute of Metrology Research Irradiator, Beijing, China, which had a Colbalt-60 source of gamma radiation, located 5 m below the surface of the platform used for radiation treatment. The gamma irradiator was reloaded in April 2012 with approximately 1.5×10^{15} Bq of cobalt-60. Reference standard dosimetry and routine dosimetry were conducted with the Fricke system. This dosimetry system was calibrated in accordance with standard ISO/ASTM 51261 (2002) and ASTM E1026-13 (2002), and the uncertainty of the measured value was calculated according to ISO/ASTM 51707 (2002).

2.3.2. Gamma radiation

In the dose–response test on late eggs, the eggs on the filter paper were placed 70 cm from the gamma radiation sources, where the absorbed dose was monitored by Fricke dosimeters on the paper; the dose rate was 4.0 Gy/min. For irradiation of late fifth instars in vitro, the 50-ml cups with 50 individuals in each were also placed 70 cm from the source. The absorbed dose was monitored by placing Fricke dosimeters in the cups; the dose rate monitored was 4.2 Gy/min.

During the dose–response tests on instars in apples, 10 apples infested with the same stage of *C. sasakii* were loaded into a plastic basket (9 × 25 × 30 cm). All the baskets were placed on the platform 70 cm from the sources to be irradiated at the target doses. Halfway through the treatment, the baskets were each turned 180° to give a more uniform exposure. The absorbed doses were monitored by placing Fricke dosimeters in the bottom, middle, and top layer of the basket. The measured dose rate was 4.56 Gy/min with the dose uniformity ratio of 1.08.

In the confirmatory tests, 50 infested apples were loaded in one plastic box (33 × 20 × 23 cm), one box was selected as controls and the other 20–22 boxes were exposed to be irradiated at the target dose of 200 Gy. On each of the five treatment dates (August and November 2012, January, April and May 2013), 10–11 boxes of infested fruits were irradiated simultaneously on the platform surrounding the sources, where the boxes were placed 100 cm from the sources. Halfway through the treatment, the boxes were each turned 180° to give a more uniform exposure. Fricke dosimeters were included in every fifth box to measure dose variation.

2.4. Rearing after irradiation

After irradiation, all the irradiated and untreated (control) eggs, infested apple, and late fifth instars in vitro were placed in the rearing room for their development to adult emergence. Each filter paper with 100 eggs on it was injected into 6 apples and placed in one 3-L transparent plastic box. Late fifth instars, including those that emerged and that remained in the apples, were collected daily and placed in sawdust until the apples decomposed. The number of late fifth instars, pupae and adults in each treatment was checked 30 d after the emergence of late fifth instars.

2.5. Statistical analysis

To investigate the radiation effect on larval mortality, dose–response data on the number of late fifth instars in each dose and control were subjected to simple linear regression using Tukey model (DPS, 2010). To make comparisons of radiation tolerance between life stages, dose–response data on percentage mortality to the adult stage were arcsine transformed and subjected to linear regression and analysis of covariance (ANCOVA) by using the Tukey model (DPS, 2010; Follett, 2008; Huang et al., 2014). Data used in the regression model included any radiation dose causing mortality between 0% and 100%, and the lowest dose causing 100% mortality. For each replicate, mortality values $<100\%$ were adjusted for control mortality using Abbott's formula (Abbott, 1925). The relationship between dose and mortality in each stage was also analyzed with probit analysis using the computer program PoloPlus (LeOra Software, 2002) to estimate the minimum dose for 99% and 99.9968% (probit 9) prevention of adult emergence from emerged late fifth instars. For the confirmatory tests, the controlling efficacy associated with treating a number of insects with zero survivors is given by the equation

$$1-Pu=(1-C)^{1/n}$$

where Pu is the acceptable level of survivorship (as a proportion), C is the confidence level, and n is the number of test insects (Couey and Chew, 1986). Efficacy $(1-Pu)$ was calculated for the number of treated *C. sasakii* late fifth instars assuming the confidence level at 95%.

3. Results

3.1. Prevention of late fifth instars emergence

Emergence of late fifth instars of *C. sasakii* after irradiation was reduced as the radiation dose up to 20 Gy for eggs, 40 Gy for 1st instars, 80 Gy for 2nd instars, and 140 Gy for 3rd instars, indicating that the minimum dose to produce larval mortality increased with increasing age and developmental stage (Fig. 1). Linear regression on the number of late fifth instars emerged showed that slope was positive and significant for eggs ($F_{1,22}=40.1$, $P<0.0001$), 1st instars ($F_{1,19}=58.7$, $P<0.0001$), 2nd instars ($F_{1,19}=59.6$, $P<0.0001$), and 3rd instars ($F_{1,19}=5.4$, $P=0.0313$), indicating that mean number of late fifth instars decreased as dose increased. However, slope was not significant for 4th instars ($P=0.3168$) or 5th instars ($P=0.5139$), indicating that radiation at the dose levels used in these tests did not strongly affect 4th and 5th instars developed to late fifth instars. Thus, late instars (L_4 and L_5) are more tolerant to radiation than eggs and early instars (L_1, L_2, and L_3) when the inhibition of late fifth instars emergence is the criteria for effectiveness.

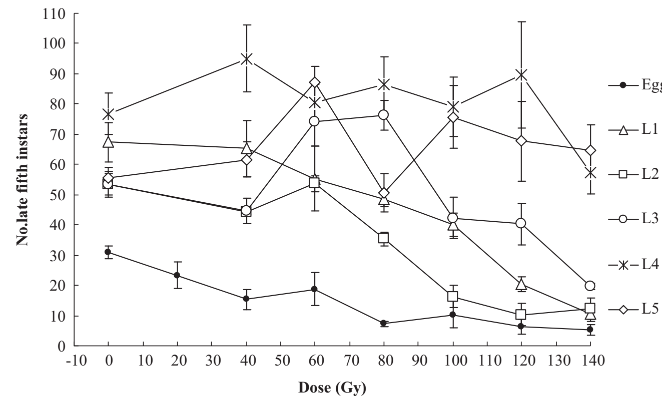

Fig. 1. Number of late fifth instars (Mean ± SE) emerged from irradiated peach fruit moth eggs and instars at all radiation doses and control (Egg: 5-d-old eggs; L1: 1st instars; L2: 2nd instars; L3: 3rd instars; L4: 4th instars; L5: 5th instars).

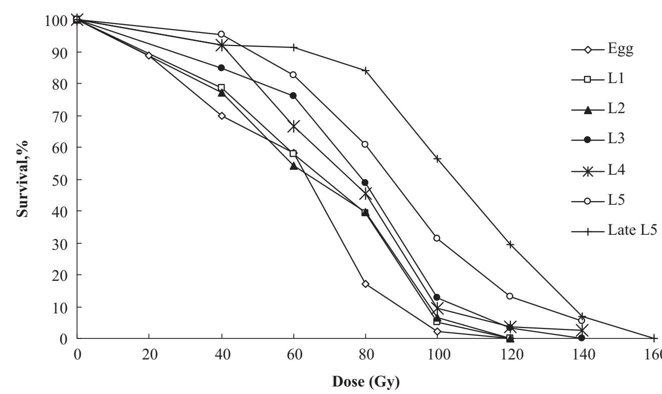

Fig. 2. Adjusted mean percentage survival to the adult stage for late fifth instars that emerged from irradiated peach fruit moth 5-d-old eggs and various instars (Egg: 5-d-old eggs; L1: 1st instars; L2: 2nd instars; L3: 3rd instars; L4: 4th instars; L5: 5th instars; Late L5: late fifth instars in vitro).

3.2. Prevention of adult emergence

An unknown number of larvae initially enter and infest the fruit after hatching from eggs, but by counting all the emerged late fifth instars as a base, the percentage survival to the adult stage at all stages and doses can be presented (Fig. 2). No late eggs or first or second instars developed to adult stage at a radiation dose of 120 Gy, no third instars developed to adult at 140 Gy, and no late fifth instars in vitro developed to adult at 160 Gy.

The dose–response data on prevention of adult emergence from emerging late 5th instars analyzed by ANCOVA showed mortality at all stages, and the radiation dose had significant interaction ($F_{13,130}=4.0$, $P=0.0001$). Therefore, all radiation doses affected the survival ability of different development stages of *C. sasakii*; the percentage mortality to the adult stage increased with increasing radiation dose. Linear regression was used to test whether slopes were significantly different from 0 (significant effect of radiation dose), and to predict a radiation dose needed to prevent adult emergence in *C. sasakii*. Slopes were positive and significant for eggs and all instars ($P<0.0001$), indicating that the tolerance to radiation increased with increasing age and developmental stage (Table 1). The 5th instars were predicted to require the highest radiation dose to prevent 100% adult emergence (estimated 164.9 Gy), whereas the egg and early larval stages were predicted to require the lowest dose to prevent 100% adult emergence (Table 1). These extrapolated values are presented to illustrate relative differences in stage-specific response and tolerance to irradiation, not to suggest a treatment dose to prevent adult emergence.

Table 1
Linear regressions on prevention of development to adult when various life stages of peach fruit moth were irradiated at 20–160 Gy.

Stage	Obs.	y-intercept ± SE	Slope ± SE	R^2	Predicted dose for 100% mortality (Gy)
Egg	18	−18.25 ± 4.77	0.92 ± 0.06	0.9342	117.3
L_1	15	−33.42 ± 5.04	1.01 ± 0.06	0.9569	122.3
L_2	15	−32.09 ± 7.01	1.01 ± 0.08	0.9201	120.7
L_3	18	−33.62 ± 5.27	0.90 ± 0.05	0.9439	137.7
L_4	18	−27.59 ± 4.99	0.81 ± 0.05	0.9398	143.4
L_5	18	−30.67 ± 3.18	0.73 ± 0.03	0.9685	164.9
L_5 in vitro	42	−39.54 ± 4.09	0.75 ± 0.04	0.9072	172.5

Table 2
Probit analysis on prevention of adult emergence when the eggs and larvae of *Carposina sasakii* were irradiated at 20–160 Gy.

Stage	No. insects	Slope ± SE[a]	Intercept ± SE[a]	ED_{99} (Gy) (95% CI)	$ED_{99.9968}$ (Gy) (95% CI)	Hetero.
Egg	244	0.039 ± 0.007	−2.326 ± 0.524	118.7 (103.6, 149.5)	161.4 (135.5, 216.3)	0.76
L_1	688	0.038 ± 0.004	−2.515 ± 0.315	126.8 (105.5, 202.7)	170.7 (134.3, 309.8)	3.29
L_2	517	0.036 ± 0.004	−2.358 ± 0.330	129.5 (106.0, 216.8)	175.8 (136.0, 331.9)	2.51
L_3	966	0.046 ± 0.005	−3.558 ± 0.423	129.2 (116.5, 156.2)	165.9 (143.6, 215.6)	1.83
L_4	1463	0.038 ± 0.002	−3.785 ± 0.223	134.0 (120.4, 159.1)	177.9 (154.4, 223.8)	3.60
L_5	1222	0.033 ± 0.002	−2.842 ± 0.258	157.5 (149.9, 167.4)	208.6 (195.0, 226.5)	0.47
L_5 in vitro	2075	0.042 ± 0.003	−4.642 ± 0.339	159.5 (149.5, 176.0)	197.8 (180.1, 228.1)	2.69

[a] Mean ± SE, CI means confidence interval, and Hetero. means Chi-square divided by degrees of freedom.

When all the dose–response data were subjected to probit analysis (without log transform of dose), the results (Table 2) showed that the relatively small value of heterogeneity (0.47–3.60) meant the estimation had good fit to the data. However, a large chi-square resulted in no prediction of effective dose (ED) for eggs, 1st, and 2nd instars by using the standard probit model (dose was log transformed) or logit or log–log model, indicating these models had poor fit to the data. The intercept decreased with increasing age except for 5th instars in apples, and then the estimated mean dose to 99% (ED_{99}) and 99.9968% ($ED_{99.9968}$) prevention of adult emergence increased with the developmental stages from eggs to fifth instars in apples, indicating the resistance to radiation in *C. sasakii* increased with increasing age and developmental stage (Table 2). Thus, the 5th instars (15-d-old larvae) likely to be found in fruits was determined to be the most tolerant stage in fruits and the minimum absorbed dose of 208.6 Gy was estimated to prevent 99.9968% adult emergence at 95% confidence level. The late 5th instars in vitro represented similar raditolerance with 5th instars in apples, as the estimated dose values for 100% mortality (172.5 vs. 164.9 Gy) and ED_{99} (159.5 vs. 157.5 Gy) were very close (Tables 1 and 2).

3.3. Confirmatory tests

Confirmatory tests were conducted to validate the estimated dose to prevent 99.9968% of adult emergence from *C. sasakii* late fifth instars, where a target dose of 200 Gy was applied to the 15-d-old instars (the most resistant stage) in apples. The result (Table 3) showed that no normal-looking adults emerged and only 4 deformed adults that failed to expand their wings emerged from a total of 30,850 late fifth instars, whereas adult emergence in the control ranged from 88.1% to 93.2%. Actual absorbed doses measured by dosimetry ranged from 171.6 to 227.8 Gy (Table 3), and the dose uniformity ratio (maximum/minimum) was 1.14 to 1.26.

4. Discussion

Tolerance to radiation in *C. sasakii* increased with increasing age and developmental stage when comparing the efficacy of preventing the emergence of late fifth instars or adults. This is in agreement with the extensive review of the PI literature by Hallman et al. (2010) who suggest that determining the most radiotolerant stage testing may not be necessary because the most developed stage is invariably the most radiotolerant when a common measure of efficacy is used. Therefore, PI researchers need only work with the most developed stage that could be present in shipped commodities.

To confirm the validity of the estimated minimum dose required to provide quarantine security, it is necessary to treat a large number of individuals of the organism while achieving the desired result, be it prevention of pest development or inability to produce offspring. The number treated will depend on the required level of efficacy (FAO, 2003, 2007; IAEA, 2009). The target dose of 200 Gy, which had achieved the same result with the related codling moth *Cydia pomonella* (L.), oriental fruit moth *Grapholia molesta* (Busck), and both apple and hawthorn colony of *C. sasakii* late fifth instars in vitro (Mansour, 2003; Hallman, 2004; Zhan et al., 2014), was applied to 15-d-old 5th instars of *C. sasakii* in apples in the confirmatory tests, resulting in no normal-looking adult emergence. Four deformed adults (0.013%) emerged from a total of 30,580 late fifth instars but they died within 2 d afterward and failed to produce eggs (Table 3). Thus, the irradiation dose (171.6–227.8 Gy measured) could provide quarantine security. Assuming the confidence level at 95%, the disinfestation efficacy calculated by the formula $1-Pu=(1-C)^{1/n}$ is 99.9902%. The maximum absorb dose used in the confirmatory tests may be the minimum dose required for the approved treatment (Heather, 2002; FAO, 2003; Hallman et al., 2010); therefore, an absorbed dose of 228 Gy (nominally 227.8 Gy) is suggested as the minimum dose for PI of *C. sasakii* in fruits.

This research supports the proposal by Hallman et al. (2013) of a generic dose of 250 Gy for all eggs and larvae of Lepidoptera on all host commodities.

When irradiation is applied on a commercial scale the maximum dose absorbed by a load can be 1.5 or 2 times the minimum dose applied (Follett and Weinert, 2009; Hallman, 2011), so that when 228 Gy is sought some of the load could receive up to ~450 Gy. A number of radiation tests demonstrated that most temperate fruits (apple, peach, nectarine, pear, plum, cherry, and

Table 3
Results of confirmatory tests of prevention of adult emergence from irradiated apples infested with 15-d-old fifth instars of *Carposina sasakii*.

Date of irradiation	No. infested apples	Target dose (Gy)	Monitored doses (Gy)	No. late 5th instars	No. adult	
					Normal-looking	Deformed
Aug. 2012	1100	200	182.4–207.5	7421	0	4[a]
	50	Control	0	404	349	8
Nov. 2012	1,000	200	185.7–227.8	4951	0	0
	50	Control	0	338	302	6
Jan. 2013	1000	200	173.5–209.2	4865	0	0
	50	Control	0	344	312	7
Apr. 2013	1000	200	171.6–205.9	5767	0	0
	50	Control	0	328	322	6
May 2013	1100	200	178.2–223.7	7576	0	0
	50	Control	0	467	388	8

[a] The adults were weak, without the extension of wings, and died within 2 d.

apricots) tolerated ≥ 500 Gy (Thomas and Romani, 1986; Zhao et al., 1987; Wang et al., 1992; ICGFI, 1994; Drake and Neven, 1998; Drake et al., 1999). This suggests that PI with the minimum absorbed dose of 228 Gy could be used as a phytosanitary treatment schedule for controlling *C. sasakii* on all commodities in international trade.

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