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Phosphine Fumigation Followed by Cold Treatment to Control Peach Fruit Moth, *Carposina sasakii*, Larvae on "Fuji" Apples Intended for Export

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Featured Application: A new application of phosphine fumigation with cold treatment to control peach fruit moth larvae *Carposina sasakii*.

Abstract: The fumigation of apples using methyl bromide (MeBr) can cause severe deterioration in fruit quality. Moreover, maintaining the quality of apples during postharvest storage and eradicating pests, especially those involved in quarantine issues, are important for facilitating the export of apples, including the "Fuji" apple (*Malus pumila* var. "Fuji") in South Korea. In the present study, phosphine (PH_3) fumigation as an alternative to MeBr was found to be more effective for the control of peach fruit moth larvae (*Carposina sasakii*), which had naturally infested Fuji apples, at a high temperature (25 °C) rather than at a low temperature (5 °C). To achieve the industry requirement of better-quality perishable commodities and meet quarantine guidelines for export, PH_3 fumigation at the low temperature (5 °C) was followed by cold treatments at 3 ± 2 °C for 2 and 4 weeks, which led to higher efficacy than was achieved using PH_3 at 5 °C alone. Given that chemical treatments, such as treatment with 1-methylcyclopropene, can inhibit ethylene synthesis, low-temperature PH_3 fumigation for 72 h followed by 4 weeks of cold treatment could also extend the shelf life of apples and resolve known quarantine issues when used as an alternative to MeBr treatment.

Keywords: phosphine fumigation; cold treatment; peach fruit moth; Fuji apple export

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1. Introduction

The peach fruit moth, *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae), is an important quarantine pest of "Fuji" apples (*Malus pumila* var. "Fuji"), which are exported from the Republic of Korea to many other countries [1]. *C. sasakii* adults lay eggs on fruits such as apple and peach, and larvae bore into the fruits and feed on the fruit flesh [2]. Each year, the apple export industry is required to demonstrate that *C. sasakii* has not been introduced to apple orchards and has been completely eradicated during quarantine and pre-shipment (QPS). Fuji apples are typically subjected to low temperatures (1.1 ± 0.6 °C) for 4 weeks during storage, after which they are treated with 48 g m^{-3} of methyl bromide (MeBr) during 2 h of fumigation [3]. Although this method effectively controls *C. sasakii* on apples in terms of quarantine regulations, MeBr fumigation can cause phytotoxic damage to

apples by softening the fruit and altering its flavor. Such effects are dependent on the apple variety and concentration of MeBr used [4]. Previous research has examined alternatives to MeBr fumigation, such as the Controlled Atmosphere Temperature Treatment System [5], ionizing radiation [1], and infrared irradiation [6]. Cold treatment can also be applied to apples as it helps control pests and extend the fruit shelf life; however, this can be a lengthy treatment depending on the target pest, developmental stage of the insect, and the potential for negative chilling injury [7]. Chemical fumigation with ethyl formate has been shown to be useful for disinfestation of the Eucalyptus weevil (*Gonipterus platensis* Marelli), which is a "hitchhiking" pest on export apples in Australia [8]. In addition, phosphine (PH_3) is known to be one of the most economic and convenient fumigants for insect pest control in grain stored in silos [9]. Therefore, fumigation with gaseous PH_3 is a practical alternative to MeBr when applied to perishable commodities such as fruits and nursery plants [10,11]. However, the use of PH_3 has some limitations. For instance, the fumigation period can be high (>24 h), depending on the temperature and target pest [11]. At lower temperatures, PH_3 fumigation must be conducted for longer periods to control the larvae of coding moths (*Cydia pomonella* L.) and peach fruit moths [12,13]. In an attempt to increase toxicity, to control the larval stage at a low application temperature, and to reduce fumigation time, PH_3 has been applied under O_2-rich atmospheric conditions (>60%), which reportedly affected the light brown apple moth (*Epiphyas postvittana* Walker) [14].

In terms of practical fumigation and market access, the application of PH_3 gas represents a simpler method compared with the currently recommended use of MeBr. Additionally, PH_3 gas should not markedly affect the quality of export apples, and it could be applied in conjunction with current postharvest treatments used to extend the shelf life in postharvest apples. Chemical treatments, such as treatment with 1-methylcyclopropene (1-MCP), can inhibit ethylene synthesis with or without cold treatments and are widely used on apple varieties postharvest [15]. Herein, we evaluated the efficacy of PH_3 gas as an alternative to current MeBr application for the eradication of *C. sasakii* larvae. PH_3 was applied at high (25 ± 1 °C) and low (5 ± 1 °C) temperatures, which was followed by 2 or 4 weeks of cold treatment (3 ± 2 °C) and the subsequent assessment of Fuji apple quality parameters.

2. Materials and Methods

2.1. Fumigants

A cylinderized gaseous mixture of PH_3 and CO_2 (ECO_2FUME^{TM}; Cytec Industries, Baulkham Hills, Australia) was used in all trials to compare the efficacy and phytotoxicity with the currently recommended MeBr (Nonghyup Chemical Co. Ltd., Seongnam-si, Korea).

2.2. Insects and Apple Fruits

The apple fruits used in efficacy tests were collected from pesticide-free orchards in Youngcheon County, Korea, in early July in each year from 2010 to 2012. Apples measured 5–6 cm in diameter and most were irregularly shaped, indicating *C. sasakii* infestation [16].

Collected apples were washed in a solution containing 0.1% methylparaben to prevent storage disease, after which they were air-dried and stored at room temperature prior to fumigation. Apples purchased from a local market served as a control when assessing the quality of fruits fumigated with PH_3 and MeBr.

2.3. Efficacy of PH_3 Alone and PH_3 Followed by Cold Treatment against Peach Fruit Moth Larvae

The first efficacy test on apples naturally infested with *C. sasakii* larvae was conducted in a 1 m^3 metal chamber, with an interior fan used for aeration. The apples were repartitioned evenly based on weight per treatment and average number of fruits. The average number of fruits per treatment was 98 and 217 apples in the 25 and 5 °C fumigation assays, respectively. Apples were fumigated with 0.5, 1.0, and 2.0 g m^{-3} PH_3 (equivalent to 25, 50, and 100 g m^{-3} of ECO_2FUME^{TM}), which can cause CO_2 levels to reach 1–5% during fumigation. After fumigation, the metal container was ventilated to decrease the concentration

of gas, and the fumigated fruits were then transported and stored at 25 ± 1 °C, 80% ± 5% relative humidity, for 14 days. In the second test, the efficacy of 2 g of PH_3 applied for various fumigation times at a low temperature (5 ± 1 °C) was evaluated. Apple samples were prepared in the same manner described for the previous bioassay.

To evaluate the effects of PH_3 fumigation followed by cold treatment, following 2 g of PH_3 fumigation (equivalent to 100 g m^{-3} of ECO_2FUME^{TM}) for 72 h, the fumigated fruits were stored at 3 ± 2 °C and 80% ± 5% relative humidity for either 2 or 4 weeks. After all treatments ended, the live larvae present in apples with and without fumigation were recovered via dissection, and their rate of survival was evaluated. Moribund larvae were held with immature apples and observed weekly to determine the rate of delayed mortality.

2.4. Measuring Fumigant Concentration and Determining the Product of C_t (Concentration × Time)

During fumigation, the concentration of PH_3 gas was determined using gas chromatography in a system (HP6890) equipped with a nitrogen–phosphorous detector. The oven temperature was 240 °C, whereas the injector and detector temperatures were both 320 °C. After separation in an HP-5 column, 1-MCP was measured using GC-FID (J&W Sci. 19091J-413). The oven temperature was 150 °C, whereas the injector and detector temperatures were both 240 °C. The gas chromatography oven was programmed as follows: start at 30 °C and increase to 100 °C at 10 °C min^{-1}, injector temperature at 150 °C, detector temperature at 250 °C, and carrier gas helium at 0.8 mL min^{-1}. The PH_3 concentration was calculated by comparing the peak area to an external PH_3 standard. The concentrations of the fumigant were monitored at intervals of 0, 24, 48, 72, 96, 120, 144, and 168 h depending on the PH_3 exposure schedule. These concentrations were then used to calculate the C_t product referred to in the previous report [17].

2.5. Quality Assessment of Apples Fumigated with PH_3 and MeBr

The quality assessment of apples was based on two recommended PH_3 fumigation methods, which both showed potential in controlling the larvae of *C. sasakii*, and two different temperature conditions as well as the MeBr treatment currently used in the industry. To assess fruit quality, we used *M. pumila* var. Fuji fruits that had been stored for 4 weeks and were previously treated with 1-MCP (1 μL L^{-1} for 24 h). After PH_3 fumigation for 72 h at either 5 ± 2 °C or 25 ± 1 °C, and after MeBr fumigation for 2 h at 15 ± 1 °C, the fumigated apples were stored for either 4 or 8 weeks prior to the evaluation of firmness, sugar content, and internal and external color change. Firmness was measured using a fruit firmness tester (53205 Digital Fruit Firmness Tester; TR Turoni, Forli, Italy) equipped with an 8 mm steel plunger. Fruits were compressed by 6 mm at the equatorial zone at a rate of 0.5 mm s^{-1}, and the maximum number developed during the operation was recorded. Firmness was measured three times per fruit, and 10 fruits were analyzed per treatment. Results were expressed in kgf. Soluble sugar content was measured using a portable refractometer (Hand Refractometer ATC-1E; Atago Co., Tokyo, Japan). Total fruit was homogenized using a tissue homogenizer and filtered through a funnel covered with filter paper. Subsequently, 0.5 mL of the filtered liquid was dropped onto a refractometer, and the sugar content was measured by observing the scope of the refractometer. For each treatment, the sugar content of 10 apples was measured, and the results were expressed as Brix%. Surface color change was measured using a colorimeter (SpectroDens, Techkon, Königstein, Germany), which expressed color as Hunter L* a* b* values. Tape was used to mark a 10 mm circle at three points along the equatorial zone of each fruit, and the internal (breakdown and flesh browning) and external colors were measured inside and on the surface of the marked circle, respectively. Flavor evaluations of apples from the individual PH_3 and MeBr treatment groups were conducted by 10–12 staff members [18]. A hedonic scale from 1 to 9 (1 = strongly dislike, 9 = strongly like) was used for all sensory ratings.

2.6. Statistical Analysis

All data-related assessments of quality parameters, such as mass, firmness, sugar content, and surface color change, and converted mortality of *C. sasakii* were conducted using SAS [19]. Data were analyzed using one-way ANOVA and Tukey's Studentized Range (HSD) test, with significance set at $p = 0.05$.

3. Results

3.1. Efficacy of PH_3 at Two Different Temperatures

For the higher-temperature treatment (25 ± 1 °C), the efficacies of 0.5, 1.0, and 2.0 g of PH_3 fumigation applied for 72 h against *C. sasakii* larvae naturally infesting apples are shown in Table 1. At 25 °C, the C_t products of 0.5 and 1.0 g m^{-3} of PH_3 in 72 h exposures were 16.7 and 42.7 g h m^{-3}, respectively, and the treatments were not sufficient to completely kill *C. sasakii* larvae. However, after exposure to 2.0 g m^{-3} of PH_3 for 72 h, the accumulated C_t product was >80.0 and the treatment had completely killed the targeted pest. In the first efficacy test at the lower temperature (5 ± 1 °C), mortality was low (>90%) even at the highest dose of PH_3, which was nearly equal to that of the C_t product (77.2 g h m^{-3}).

Table 1. Efficacy of 72 h phosphine (PH_3) fumigation at two different temperatures in apples naturally infested with *Carposina sasakii* larvae. Different letters above symbols indicate significant differences ($p < 0.05$) as determined using a one-way ANOVA and Tukey's Studentized Range test.

Treatment	Temp. (°C)	Fumi. Time (h)	Dose (g m^{-3})	CT (g h m^{-3})	Total No. of Fruit Used	No. of Alive *C. sasakii* Larvae	No. of Alive *C. sasakii* Larvae per Fruit	Converted Mortality (%)
Untreated	5	72	-	-	87	223	2.6 ± 0.46	-
PH_3			0.5	14.1	90	155	1.7 ± 0.44	32.73 ± 17.34 a
PH_3			1.0	38.4	101	84	0.8 ± 0.12	67.56 ± 4.83 b
PH_3			2.0	77.2	117	63	0.5 ± 0.26	78.97 ± 10.17 b
Untrated	25	72	-	-	199	187	0.9 ± 0.34	-
PH_3			0.5	16.9	258	5	0.0 ± 0.01	97.94 ± 1.43 a
PH_3			1.0	42.7	208	2	0.0 ± 0.01	98.97 ± 1.78 a
PH_3			2.0	80.3	203	0	0.0 ± 0.00	100.0 ± 0.00 a

Based on a previously determined relationship between PH_3 efficacy and exposure time at low temperatures (unpublished data), the efficacies of 2.0 g m^{-3} of PH_3 at 96–168 h exposures are shown Table 2. Fumigation with 2.0 g of PH_3 for 96, 120, 144, and 168 h (equivalent to 81, 4, 118.3, 135.8, and 160.2 of accumulated C_t products, respectively) was not sufficient to eradicate *C. sasakii* larvae (Table 2).

Table 2. Efficacy of phosphine (PH_3) fumigation at 5 °C in apples naturally infested with *Carposina sasakii* larvae. Different letters above symbols indicate significant differences ($p < 0.05$) as determined using a one-way ANOVA and Tukey's Studentized Range test.

Treatment	Temp. (°C)	Fumi. Time (h)	Dose (g m^{-3})	CT (g h m^{-3})	Total No. of Fruit Used	Alive *C. sasakii* Larvae	Alive *C. sasakii* Larvae per Fruit	Converted Mortality (%)
Untreated	5	-	-	-	27	40	1.4 ± 0.39	-
PH_3		96	2.0	81.4	27	5	0.2 ± 0.16	90.69 ± 6.44 a
PH_3		120	2.0	118.3	25	3	0.1 ± 0.19	95.65 ± 7.51 a
PH_3		144	2.0	135.8	26	2	0.1 ± 0.12	97.10 ± 5.01 a
PH_3		168	2.0	160.2	27	2	0.1 ± 0.03	97.10 ± 2.50 a

3.2. Efficacy of PH_3 at Low Temperature Followed by Cold Treatment

At the lower temperature (5 ± 1 °C), prolonged exposure to PH_3 (up to 168 h) did not meet the current quarantine regulations, >99.99% (Tables 1 and 2). The efficacy results of 2.0 g of PH_3 in a 72 h fumigation followed by 2 or 4 weeks of cold treatment are shown

in Table 3. The accumulated C_t product of PH_3 was 84.3 g h m^{-3}, and *C. sasakii* larvae were completely eradicated after 4 weeks of cold storage at 3 ± 2 °C. Despite the increased mortality rate, 78.7% mortality was observed after 2 weeks and 90.5% after 4 weeks in the untreated control at 3 ± 2 °C. The unexpectedly high mortality observed in the control was expected to have played a role in the physical control of *C. sasakii* larvae.

Table 3. Effects of 72 h phosphine (PH_3) fumigation at 5 °C followed by 2- and 4-week cold treatment (3 ± 2 °C) in apples naturally infested with *Carposina sasakii* larvae. Different letters above symbols indicate significant differences ($p < 0.05$) as determined using a one-way ANOVA and Tukey's Studentized Range test.

Treatment	Fumi. Time (h)	Dose (g m^{-3})	CT (g h m^{-3})	Following Cold Treatments (Weeks)	Total No. of Fruit Used	No. of Alive *C. sasakii* Larvae	Average No. of *C. sasakii* Larvae per Fruit	Converted Mortality (%)
Untreated	-	-	-	2	31	17	0.5 ± 0.13	78.7 ± 4.97 a
				4	35	5	0.2 ± 0.16	90.5 ± 5.42 ab
PH_3	72	2.0	84.3	2	30	2	0.1 ± 0.11	97.4 ± 4.51 b
				4	32	0	0.0 ± 0.00	100.0 ± 0.00 b

3.3. Assessment of Quality Parameters in Apples Treated with PH_3 and MeBr Fumigation Followed by Cold Treatment

After being stored for either 4 or 8 weeks at 3± 2 °C, respective firmness was 1.57 ± 0.29 and 1.64 ± 0.06 kgf for untreated fruits, 1.64 ± 0.23 and 1.66 ± 0.07 kgf for 120 h PH_3 fumigation at 5 ± 2 °C, 1.60 ± 0.16 and 1.62 ± 0.08 kgf for 72 h PH_3 fumigation at 25 ± 2 °C, and 1.43 ± 0.21 and 1.64 ± 0.07 kgf for 2 h MeBr fumigation, respectively. There was no significant difference in firmness among the four treatments, including untreated samples that were stored for four weeks (df = 24, $p = 0.400$) or eight weeks (df = 24, $p = 0.874$). Additionally, there was no significant difference in sugar content in the apples from the four treatments stored for four weeks (df = 16, $p = 0.415$) or eight weeks (df = 16, $p = 0.195$). In relation to the weight of untreated samples, the ratio of weight loss of PH_3-treated apple was not significantly different when stored for 4 weeks (df = 16, $p = 0.942$), but there was a significant difference of the weight loss ratio among samples stored for 8 weeks (df = 16, $p = 0.012$). Apples treated with MeBr exhibited the greatest amount of weight loss, followed by those treated with PH_3 and untreated samples (Table 4). Both the internal and external color of fruit changed after storage for 4 weeks, but there were no significant differences between the treated and untreated samples in their internal color (df = 24, $p = 0.405$) or external color (df = 16, $p = 0.904$). However, after storage for 8 weeks, MeBr treatments showed differences in both internal (df = 16, $p = 0.405$) and external (df = 16, $p < 0.001$) color change. Significant differences were observed in the index of flavor scores in terms of aroma and taste. Of all treatments, apples that were subjected to exposure to MeBr and subsequent 4- or 8-week storage received the lowest sensory ratings. Relative to untreated samples, 2.0 g m^{-3} of PH_3 applied for 72 h at either 5 ± 1 °C or 25 ± 1 °C had no negative effects on the fruit in terms of firmness, sugar content, weight loss, color change, and flavor. However, MeBr fumigation had negative effects in terms of color change, aroma, and taste.

Table 4. Phytotoxicity assessment in apples fumigated with phosphine (PH_3) and methyl bromide (MeBr) fumigations followed by 4- and 8-week cold treatments (3 ± 2 °C). Different letters above symbols indicate significant differences ($p < 0.05$) as determined using a one-way ANOVA and Tukey's Studentized Range test.

Treatment	Fumi. Temp (°C)	Fumi. Time (h)	Dose (g m^{-3})	CT (g h m^{-3})	Following Cold Treatments at 3 ± 2 °C (Weeks)	Firmness ± SE (kg f)	Sugar Content ± SE (%)	Initial Average Weight ± SE (g)	Ratio of Weight Loss ± SE (%)	Color Change		Flavor Score	
										Internal	External	Aroma	Taste
Untreated	-	-	-	-	4	1.57 ± 0.29 a	12.56 ± 1.01 a	334.40 ± 6.84 a	0.94 ± 0.11 a	79.97 ± 0.48 a	51.46 ± 1.60 a	7.4 ± 0.9 a	6.3 ± 1.2 a
PH_3	5	120	2.0	121.5	4	1.64 ± 0.23 a	12.64 ± 1.08 a	334.71 ± 4.74 a	0.84 ± 0.39 a	78.68 ± 0.57 a	52.03 ± 1.76 a	7.5 ± 0.6 a	6.5 ± 1.2 a
PH_3	25	72	2.0	89.9	4	1.60 ± 0.21 a	13.28 ± 0.70 a	332.76 ± 6.05 a	0.94 ± 0.29 a	78.68 ± 0.51 a	51.91 ± 1.59 a	7.6 ± 0.8 a	7.2 ± 2.0 a
MeBr	15	2	48.0	129.5	4	1.43 ± 0.21 a	12.28 ± 0.91 a	336.54 ± 5.36 a	0.95 ± 0.41 a	78.40 ± 1.12 a	51.63 ± 1.65 a	4.8 ± 1.4 b	4.4 ± 1.1 b
Untreated	-	-	-	-	8	1.64 ± 0.06 A	12.44 ± 0.82 A	331.33 ± 7.13 A	1.85 ± 0.19 B	78.90 ± 0.87 A	51.55 ± 1.66 A	6.0 ± 1.0 A	6.1 ± 1.1 A
PH_3	5	120	2.0	121.5	8	1.66 ± 0.07 A	13.24 ± 0.07 A	329.96 ± 5.04 A	2.25 ± 0.56 A,B	78.98 ± 0.66 A	52.20 ± 1.60 A	7.0 ± 2.0 A	6.1 ± 1.6 A
PH_3	25	72	2.0	89.9	8	1.62 ± 0.08 A	12.22 ± 0.92 A	328.93 ± 6.01 A	2.08 ± 0.29 A,B	78.39 ± 1.53 A	51.98 ± 1.35 A	7.2 ± 2.0 A	6.9 ± 1.8 A
MeBr	15	2	48.0	129.5	8	1.64 ± 0.07 A	12.22 ± 0.79 A	330.41 ± 5.37 A	2.76 ± 0.38 A	66.64 ± 11.07 B	41.35 ± 3.18 B	3.6 ± 1.1 B	1.9 ± 0.9 B

4. Discussion

To meet QPS regulations in Korea, we suggest that fumigation with PH_3 could be used as a viable alternative to MeBr treatment. Unlike MeBr, PH_3 effectively kills peach fruit moth larvae in Fuji apples while maintaining fruit quality according to the parameters measured in this study. Soma (2000) reported a study on the comparison between MeBr and a 24 h treatment of PH_3 in which PH_3 did not induce apparent damage on pear but was not efficient to control *C. sasakii* [20]. In our study, 72 h treatment of PH_3 was required even at higher temperatures, but no damage was observed on apple. At lower temperatures, Liu et al. (2010) reported that a longer period is required to control *Carposina niponensis* with PH_3 [13]. Our study also supports this result, in which an 168 h treatment was not sufficient to eradicate *C. sasakii* larvae at 5 °C. Although MeBr fumigation in apples is more time- and cost-effective compared with PH_3 fumigation, the MeBr residue on the fruit may be of concern; therefore, for natural reduction of bromide, a longer storage period at a lower temperature (>13 days at 2 °C following 2 h MeBr fumigation) might be required [21]. Besides, PH_3 fumigation normally leaves lesser residues that rapidly decreased after ventilation [22]. Phytotoxic damage, such as that shown by internal disorder and low flavor scores, was examined in "Delicious" and "Spartan" apple varieties after the recommended 48 g m^{-3} MeBr fumigation for 2 h [4]. Depending on the apple variety, MeBr fumigation causes injuries such as water-core and internal breakdown in Fuji apples [23], and these apples exhibit similar symptoms in terms of changes to internal color and flavor. Although the same apple cultivar, "Fuji", was used in the present study, there might have been slight differences in terms of cultivation history and postharvest 1-MCP treatment. However, the identical scheduled MeBr fumigations elicited similar phytotoxic symptoms, such as softening and off-flavoring, in the current work. Fuji apples treated with 1-MCP, which was used to assess fruit quality in this study, show inhibition of ethylene synthesis with PH_3 treatment (unpublished data). It is expected that positive results could be yielded by commercial use of 1-MCP treatment followed by PH_3 fumigation in Fuji apples because, at the time of harvest in Korea, most apples are treated with 1-MCP prior to cool storage. Even though the cold treatment itself increased the shelf life of fruit and resulted in increased mortality in *C. sasakii* larvae (up to 90% for 4 weeks of storage at 3 ± 2 °C) in the present study, the treatment has limited use in QPS. Unlike metal phosphide fumigation, 72 h PH_3 gas fumigation can be considered as an alternative to MeBr fumigation for various perishable commodities that are stored at temperatures from −1.5 to 2 °C [10]. Although the efficacy of PH_3 fumigation against *C. pomonella* larvae was not sufficiently demonstrated [10], we demonstrated its efficacy against *C. sasakii* larvae in terms of insect mortality. Although limitations exist with PH_3 treatment in terms of temperature and time, PH_3 gas could act as a replacement for MeBr for the fumigation of apples exported from Korea because the former had no negative effects on the quality of Fuji apples. Due to the low negative effect on fresh commodities, more studies about PH_3 gas are processed to replace MeBr for quarantine purposes [24,25]. Further studies are necessary to examine the relationship between PH_3 fumigation and low-temperature treatment in terms of the effects against *C. sasakii*, and such studies should ideally focus on demonstrating efficacy on a commercial scale.

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