

A REFEREED PAPER

## AN ASSESSMENT OF METHODS TO CLEAN CITRUS FRUIT SURFACES

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**Abstract.** A move away from harsh chemicals towards more natural or organic postharvest treatments for sanitizing fruit surfaces has prompted interest in alternate sanitizers for cleaning fruit in packinghouses. In this study a comparison was made of some sanitizing methods presently used (e.g. warm water and sodium hypochlorite solutions) with the sanitizing activity of peroxyacetic acid. To assess the initial microflora population on the surfaces of unwashed fruit, oranges were collected from the field and their surfaces washed in sterile buffer solutions. The buffer was analyzed for numbers of microbial organisms, and populations were found to be between approximately  $\log_{10}$  4.0 and  $\log_{10}$  6.0 cfu/cm<sup>2</sup> of fruit surface. Organisms from these analyses were isolated for later use. To assess the effectiveness of sanitizers on microbial populations on the fruit surfaces, oranges were picked from the field and surface sterilized in a circulating hot water bath (2 minutes at 85 °C). A cocktail of microorganisms previously isolated from the surfaces of oranges was made. These included the pathogens *Penicillium digitatum*, *Geotrichum citri-aurantii* and *Colletotrichum gloeosporioides*. In addition, 2 non-pathogenic yeasts and a bacterium were included to obtain a  $\log_{10}$  6.0 total concentration of suspended spores and cells. The sterilized oranges were inoculated by dipping them into this spore mix for 30 seconds. The fruit were allowed to air dry for 24 hours and were then sanitized with warm water, sodium hypochlorite (200 ppm, pH 6.5) or peroxyacetic acid (100 ppm). Averaged microflora populations remaining on orange surfaces after treatments were; warm water  $\log_{10}$  4.1 cfu/cm<sup>2</sup>, sodium hypochlorite  $\log_{10}$  3.3 cfu/cm<sup>2</sup> and peroxyacetic acid  $\log_{10}$  2.4 cfu/cm<sup>2</sup>.

Increases in the consumption of fresh produce and consumer concern for healthy foods have necessitated changes in the manner in which these commodities are produced, transported, processed, stored and marketed (Buck and Walcott, 2003). Larger volumes of produce sales amplify the amount of postharvest decay. Microbial contamination of produce can be attributed to many factors, including variety and maturity, field practices (e.g., irrigation, fertilizing, handling by workers), contaminated bins and equipment, and transport vehicles (Brackett and Splittstoesser, 2001; Buck and Walcott, 2003; DeRoever, 1998; Hobbs, 1986; Waller, 2002). Packing, storage and shipping operations can contribute microorganisms to produce surfaces especially with improper sanitation practices (DeRoever, 1998). Some organisms causing post-

harvest decay are in contact with the fruit while it is still in the field and remain undetected until the fruit has been processed and or shipped (Skaria et al. 2003; Waller, 2002).

Citrus shippers often indicate concerns about fruit quality problems at delivery, which include bruising, rots, or a combination of problems that were not visible at the time of shipping. These result in unmarketable fruit. In a comparison study between field packed (unwashed) and packinghouse (processed on packing lines) fruit, field packed fruit had significantly less bruising and decay than fruit that had been cleaned and processed through a packing line (Skaria et al., 2003). Microbial populations on the surfaces of field fruit include non-pathogenic organisms which can compete with pathogens for food and space. When these competitive organisms are removed by washing, pathogens have more opportunity to grow (Skaria et al., 2003).

Fruit that has been injured during harvest or processing is often invaded by wound pathogens such as *Penicillium digitatum* Link, *Penicillium italicum* Link and *Geotrichum citri-aurantii* Link, which destroy the fruit in storage or transit (Ismail and Zhang, 2004; Skaria et al., 2003). Many times these pathogens are picked up in packinghouses where they are found on discarded fruit, and can make their way back onto the packing line after the fruit has been initially cleaned (Ismail and Zhang, 2004; Skaria et al., 2003). Good packing line sanitation reduces the inoculum density on fruit surfaces by both cleaning the spores from the line and the surfaces of the fruits.

Some postharvest decay results from organisms that have been on the fruit preharvest and become active as the fruits mature or are degreened (Ismail and Zhang, 2004). These organisms, such as *Lasiodiplodia theobromae* Ellis & Everh., *Phomopsis citri* (Sacc.) Brubák, *Colletotrichum gloeosporioides* Corda, *Phytophthora citricola* deBary and *Alternaria alternata* pv *citri* Nees, are often not easily removed or cannot be removed from the fruit and are frequently not detected during the cleaning and the packing process.

Currently, cleaning fruit and removing surface grime (which harbors microbes including post harvest pathogens) are usually achieved by spraying a sanitizer (such as chlorine) in water and washing on brushes followed by a potable water rinse (Ismail and Zhang, 2004). However, limitations of cleaning with chlorine include constant adjustment (topping off) of the available chlorine in sanitizing flumes or sprays, necessity of a rinse with clean water (chlorine releasing compounds can produce halogenated by-products when reacting with organic materials) (Taverner, 2004) and monitoring of pH. Also, many microorganisms are not sensitive to chlorine rinses, especially those in high populations or those encased in biofilms (Sapers, 2001).

Like chlorine washes, peroxyacetic acid (PAA) reacts with and breaks down organic material; however, it is not as sensitive as chlorine and the rate of loss of PAA from solution when exposed to organic material is less pronounced (Taverner, 2004). Decomposition products of PAA are acetic acid and oxygen, which are not toxic and no rinse step is necessary (Taverner, 2004). PAA is effective against a wide range of

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microorganisms and is available for a longer time in solution than chlorine, thus increasing contact time with surface microflora and yielding more consistent sanitizing.

Sanitizers can be commodity specific: i.e., some will work more efficiently than others on various types of peel topography. In a comparison study of different methods of sanitizing whole fruit citrus, we reduced microbial populations on orange surfaces using warm water, chlorine and a commercial peroxyacetic acid solution, and compared their efficiencies in removing these organisms.

### Materials and Methods

**Field fruit assessment.** Using sterile gloves, twenty-five oranges (*Citrus sinensis*) were picked from the State of Florida Arboretum grove at Lake Fanny in Winter Haven and taken to the USDA Citrus and Subtropical Products Laboratory. (Ripe and near ripe fruit were picked and analyzed in the fall (Pineapple oranges) and spring (Valencia oranges). Each fruit was placed in a sterile Whirl-Pak bag (Nasco, Modesto, Calif.) containing 99 mL of sterile phosphate buffer (pH 7.2). Every fruit was "washed" for 2 min by rubbing and agitating the fruit through the bag (Method modified from Parnell and Harris, 2003, Smilanick et al., 2003). After washing, fruits were removed from the bags and left to air-dry before measuring with a digital caliper (Control Company, ISO 9001 Cert., Friendswood, Texas) to allow surface areas to be determined. As measurements of fruits in two planes resulted in a spheroid shape, the formula for the surface area of a sphere ( $4\pi r^2$ ) was used to determine surface area ( $\text{cm}^2$ ). The buffer solutions remaining in the bags were analyzed for microorganisms by removing a 5 mL aliquot from each bag and plating on agar plates using a Whitley Automatic Spiral Plater (DW Scientific, Ltd., Shipley, UK). Samples were plated onto three types of media; potato dextrose agar (PDA), orange serum agar (OSA) and plate count agar (PCA). In the second field wash only, acidified potato dextrose agar (APDA) was substituted for OSA to see if any difference in organisms isolated was achieved as APDA is very acidic. As little growth occurred on this medium, isolations continued using OSA. (All agars were Becton Dickinson, Sparks, Md.). The PDA and OSA agars encouraged growth of yeasts and molds, while PCA was used to isolate bacteria. All plates were incubated at 35 °C for 48 h and the results (initially bacteria and yeasts) were read on a ProtoCol colony counter (Synoptics, Ltd., Cambridge, UK). After initial counts were made on the ProtoCol, plates were left at 25 °C for 5-7 d and additional filamentous fungi that appeared were added to the original microbial count. Colony counts for replicate plates were averaged together, adjusted with surface area measurements and reported as cfu (colony forming units) per  $\text{cm}^2$  of fruit surface.

**Sanitizer assessment.** Ninety oranges were collected from the grove at Lake Fanny and taken to the laboratory. The fruits were washed under warm (35 °C) tap water with gloved hands and scrubbing pads. The fruits were air dried and then placed in an 85 °C circulating water bath for 2 min for surface sterilization (Pao and Davis, 1999). The oranges were then removed with sterile tongs and placed on sterile foil sheets to dry. Five of these fruits were randomly selected to test the efficiency of the surface sterilization process by assessing surfaces as previously described.

Approximately 2.5 L of inoculum was made up using sterile phosphate buffer. Five 9 mL phosphate buffer blanks were

inoculated with cells and spores of five organisms that were previously isolated from the field washes of orange surfaces. These isolates were grown on appropriate media (PDA for fungi and PCA for bacteria) and spores or cells were aseptically harvested and re-suspended in a sterile buffer solution. These organisms included a gram + bacterium, *Rhodotorula* spp. F. C. Harrison, *Colletotrichum gloeosporioides*, *Penicillium digitatum* and *Geotrichum citri-aurantii* in an approximately  $\log_{10}$  6.0/mL suspended cell concentration for all repeated runs. Spore concentration was the sum representation of all organisms and was calculated with a hemacytometer (Hausser Scientific, Horsham, Pa.).

With sterile tongs, the dry, surface sterilized fruits were inoculated by holding them in the inoculum cocktail for 30 s. A sterile stir bar kept the inoculum in suspension as the fruits were dipped. The fruits were placed on sterile foil sheets and allowed to air dry for 24 h. After the 24 h drying period, 10 of the inoculated fruits were randomly selected and used as a control group (inoculated but not sanitized). They were assayed in Whirl-Pak bags as previously described. The remaining 75 fruits were divided into 3 groups of 25 fruits and used for the sanitation study.

Three 5-gallon plastic pails were sanitized with a weak bleach solution and rinsed several times with distilled water and allowed to dry. The clean pails were then used to hold 10 L of experimental sanitizing liquid: warm tap water (35 °C), sodium hypochlorite (NaOCl) (200 ppm acidified to pH 6.5 with a 2 M solution of citric acid) and a commercial peroxyacetic acid solution (PAA) (100 ppm, pH 3.2) (StorOx, Bio-Safe Systems, Glastonbury, Conn.). Six to seven fruits were washed at a time. The fruits were held under the surface of the solutions with gloved hands and gently rubbed and agitated for 1 min. They were removed and placed on sterile foil under a laminar air flow hood to dry. The pails were cleaned and filled with fresh solution after every 6-7 fruits. The fruits were allowed to air dry and then were assessed for any microorganisms remaining on their surfaces using the assay previously described. The experiment was repeated three times; twice with fruits picked in the fall (Orange 1 and 2) and once with fruits picked in the spring (Orange 3).

**Statistical analysis.** The Kolmogorov-Smirnov Test for Different Distributions (Number Cruncher Statistical System, Kaysville, Utah) was used to evaluate fruit sanitizer data (pair wise comparison): warm water or NaOCl versus the peroxyacetic acid solution.

### Results and Discussion

**Field fruit assessment.** Results from the field washes show a difference in microflora populations on the surfaces of oranges from the same grove at different times of the year (Figs. 1 and 2). Data taken from fruits picked in late fall (November) had counts of  $\log_{10}$  3.7 cfu/ $\text{cm}^2$  on OSA and PDA, and  $\log_{10}$  3.8 cfu/ $\text{cm}^2$  on PCA (Fig. 1). A second fall harvest of the same fruit variety and maturity showed similar results. Fruits assessed from the same grove area the following spring (March) resulted in microbial counts higher than the previous fall;  $\log_{10}$  5.8 cfu/ $\text{cm}^2$ ,  $\log_{10}$  5.5 cfu/ $\text{cm}^2$  and  $\log_{10}$  3.9 cfu/ $\text{cm}^2$  on PCA, PDA and APDA respectively (Fig. 2). The dynamic character of microflora populations on fruit surfaces is well documented (Brackett and Splittstoesser, 2001; Garg et al., 1990; Hobbs, 1986). Fluctuations in these populations occur on a seasonal basis as well as varying with the maturity of the fruits,

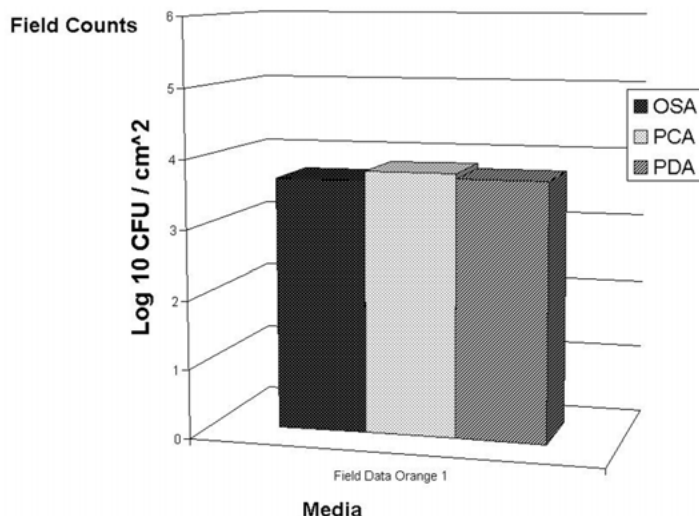


Fig. 1. Microbial counts from orange fruit surfaces from fruits harvested in late fall (November). Counts are replicate plates averaged together.

the overall health of the tree and placement of the fruits on the tree. Systems to clean fruit surfaces must take into account these variable concentrations of organic materials which affect the efficiency of some sanitizing solutions.

**Sanitizer assessment.** Data from fruit that were inoculated and sanitized with warm water, NaOCl (200 ppm) or PAA (100 ppm) showed differences in the efficiencies of these methods (Figs. 3-5). Seasonal differences in surfaces or peel changes during surface sterilization might account for the variation in numbers of microorganisms adhering to the orange surface during inoculation. For Orange 1 (first inoculation), control (fruits inoculated but not sanitized) had  $\log_{10}$  4.7 cfu/cm<sup>2</sup> on OSA and PCA and  $\log_{10}$  4.8 cfu/cm<sup>2</sup> on PDA (Fig. 3). Inoculated fruits from Orange 1 sanitized with warm water had surface colonies reduced to  $\log_{10}$  3.8 cfu/cm<sup>2</sup>,  $\log_{10}$  4.1 cfu/cm<sup>2</sup> and  $\log_{10}$  4.0 cfu/cm<sup>2</sup> on OSA, PCA and PDA, respectively, while fruit surfaces sanitized with NaOCl had final microbial populations of  $\log_{10}$  3.3 cfu/cm<sup>2</sup> on OSA and PCA and  $\log_{10}$  3.5 cfu/cm<sup>2</sup> on PDA (Fig. 3). Fruit surfaces sanitized

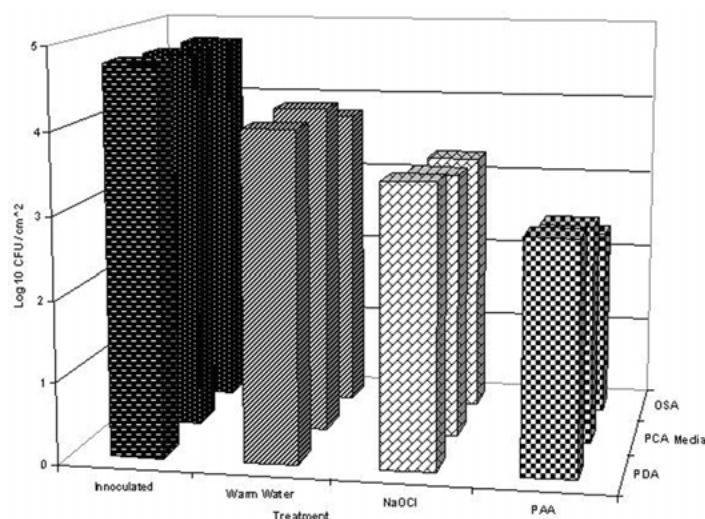


Fig. 3. Microbial counts from Orange 1 (first inoculation trial (fall fruit)): counts are replicate plates averaged together. "Inoculated" are control fruits (inoculated but not sanitized).

with PAA in Orange 1 had significantly fewer microorganisms than those treated with warm water ( $P < .001$ ) or NaOCl ( $P < .01$ ), with population counts of  $\log_{10}$  2.3cfu/cm<sup>2</sup>,  $\log_{10}$  2.7 cfu/cm<sup>2</sup> and  $\log_{10}$  2.9 cfu/cm<sup>2</sup> on OSA, PCA and PDA.

For both Orange 2 and 3 (repeated inoculation trials), the results were similar to Orange 1: the PAA reduced the microbial populations on the fruit surfaces more efficiently than either warm water or NaOCl (Figs. 4 and 5).

In Orange 2, control fruits (inoculated and not sanitized) had microbial populations of  $\log_{10}$  3.9 cfu/cm<sup>2</sup> (OSA and PCA) and  $\log_{10}$  4.0 cfu/cm<sup>2</sup> (PDA) (Fig.4). Reductions in microbial populations from a warm water rinse were  $\log_{10}$  3.2 cfu/cm<sup>2</sup> (OSA) and  $\log_{10}$  3.3 cfu/cm<sup>2</sup> (PCA and PDA), while NaOCl treated fruits had final microbial populations of  $\log_{10}$  2.0 cfu/cm<sup>2</sup> (OSA) and  $\log_{10}$  2.1 cfu/cm<sup>2</sup> (PCA and PDA). Those fruits sanitized with PAA in Orange 2 had surface microbial populations of  $\log_{10}$  1.5 cfu/cm<sup>2</sup> (PCA) and  $\log_{10}$  1.4

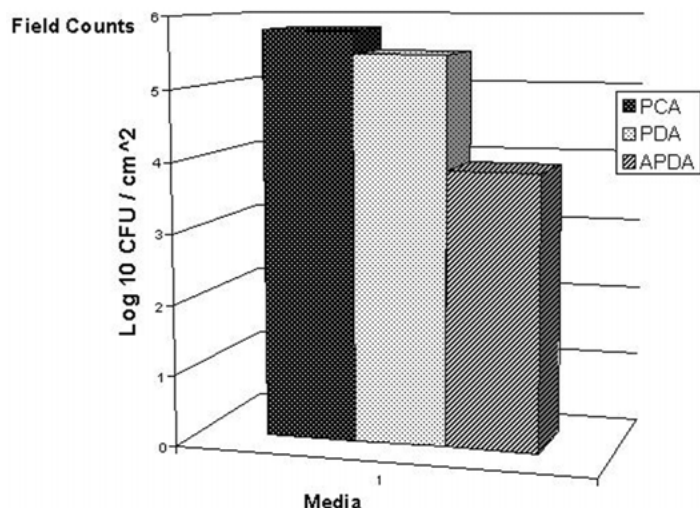


Fig. 2. Microbial counts from orange fruit surfaces from fruits harvested in the spring (March). Counts are replicate plates averaged together.

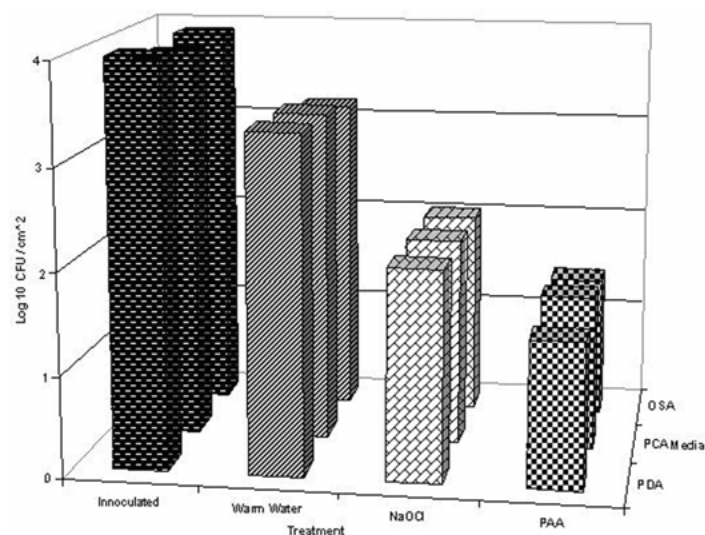


Fig. 4. Microbial counts from Orange 2 (second inoculation trial (fall fruit)): counts are replicate plates averaged together. "Inoculated" are control fruits (inoculated but not sanitized).

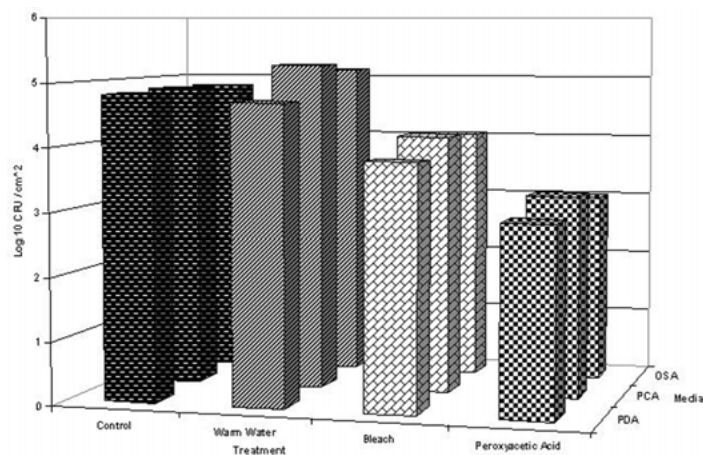


Fig. 5. Microbial counts from Orange 3 (third inoculation trial (spring fruit)): counts are replicate plates averaged together. "Inoculated" are control fruits (inoculated but not sanitized).

cfu/cm<sup>2</sup> (OSA and PDA) (Fig. 4), a significant reduction over warm water ( $P < .001$ ) and NaOCl ( $P < .001$ ) results.

Control fruits for Orange 3 had microflora populations of log<sub>10</sub> 4.8 cfu/cm<sup>2</sup> on OSA, PCA and PDA respectively (Fig. 5). Microbial populations for Orange 3 fruits washed with warm water were log<sub>10</sub> 5.1, 5.2 and 4.7 cfu/cm<sup>2</sup> on OSA, PCA and PDA respectively, while for fruit sanitized with NaOCl, reductions were log<sub>10</sub> 4.0, 4.1 and 3.8 cfu/cm<sup>2</sup> on OSA, PCA and PDA. Microflora populations on fruits in Orange 3 sanitized with PAA were log<sub>10</sub> 3.0 cfu/cm<sup>2</sup> (OSA and PDA) and log<sub>10</sub> 3.2 cfu/cm<sup>2</sup> (PCA) (Fig. 5) a significant reduction in microbial populations as compared to warm water ( $P < .001$ ) and NaOCl ( $P < .001$ ) treated fruits.

Table 1 data are a summary of microbial counts on orange surfaces after treatments. The numbers are averages of all media types and significance is shown. All counts are measured in log cfu/cm<sup>2</sup>.

### Conclusions

Data show that microbial populations on unwashed fruit surfaces can be variable with seasonal change. Florida citrus is harvested from September until May, and the microflora on these fruits changes over the duration of the harvest period. As most citrus packinghouses do not modify sanitizing systems during the harvest season, it is important that the sanitizing systems used be consistent in the reduction of surface microflora over a range of population densities.

Our studies show that, when comparing warm water, sodium hypochlorite (NaOCl, 200 ppm, pH 6.5) and peroxyacetic acid (PAA, 100 ppm), PAA consistently reduced microbial populations. Warm water used as a cleaning agent reduced microbial populations by less than 1 log. Log reductions of microbial populations for NaOCl and PAA were approximately 1.2 and 2.1 respectively. Because the efficiency of PAA is not

Table 1. Summary of microbial counts on orange surfaces after treatments: numbers are averages of all media types and significance is shown (control, n = 10; water, NaOCl and PAA each, n = 25). All counts are measured in log 10 cfu/cm<sup>2</sup>.

Treatment	Orange 1	Orange 2	Orange 3
Control	4.7	3.9	4.8
Water	4.0 *	3.3 *	5.0 *
NaOCl	3.7 ^	2.1 +	4.0 +
PAA	2.6 * ^	1.4 * +	3.1 * +

Significance shown by the Kolmogorov-Smirnov Test for Different Distributions: (\*) all water and PAA at  $P < .001$ ; (^) Orange 1 NaOCl and PAA at  $P < .01$  and (+) Orange 2 and 3 NaOCl and PAA at  $P < .001$ . Numbers are averages of replicate plates

reduced as rapidly as that of NaOCl when exposed to organic materials, a longer contact time could possibly reduce microbial populations further.

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