Evaluation of abamectin as a potential chemical control for the lychee erinose mite (Acari: Eriophyidae), a new invasive pest in Florida

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Abstract

The lychee erinose mite, *Aceria litchii* (Keifer) (Acari: Eriophyidae), is an important pest of lychee (*Litchi chinensis* Sonn.; Sapindaceae) trees. This minute mite prefers to feed on young, new flush causing the formation of galls called "erinea." Chemical control to protect the new flush is the primary management approach that has been used to control this mite. *Aceria litchii* was detected recently in Lee County, Florida, USA, and there is an urgent need to identify an acaricide that can control mite populations. Among the acaricides registered for use on lychee in Florida, abamectin was reported to be effective against *A. litchii* from other parts of the world. However, it remains unknown whether this acaricide can control the mites inside the erinea effectively and protect the new flush. We investigated whether abamectin alone or in combination with an organosilicone surfactant could control an existing mite infestation. Lychee leaflets that had erinea were sprayed with acaricides, then placed on uninfested plants and monitored for symptom development. One mo after placing treated leaflets on uninfested plants, the same treatment was applied to the whole plants and monitored for erinea development on the new flush. Our results showed that none of the treatments were able to control the mites inside the erinea and protect the new flush. The methods described here can be used for more precise evaluations of other acaricides that are urgently needed to control *A. litchii* in Florida.

Key Words: acaricide; organosilicone surfactant; *Aceria litchii*; erineum; quarantine

Resumen

El ácaro erinoso del litchi, *Aceria litchii* (Keifer) (Acari: Eriophyidae) es una plaga importante de árboles de litchi (*Litchi chinensis* Sonn.; Sapindaceae). Este ácaro diminuto prefiere alimentarse de tejidos jóvenes e induce la formación de un tipo de agallas llamadas "erineo." El control químico para proteger las hojas jóvenes es el método más utilizado para controlar este ácaro. El ácaro erinoso del litchi fue detectado recientemente en el condado de Lee, Florida, USA, por lo cual se requiere urgentemente identificar acaricidas para controlarlo. Dentro de los acaricidas registrados para el uso en litchi en Florida, la abamectina ha sido reportado como eficiente para el control de este ácaro en otras partes del mundo. Sin embargo, se desconoce si este acaricida puede controlar al ácaro dentro del erineo y proteger los tejidos jóvenes. Investigamos si abamectina sola o en combinación con un tensoactivo organosiliconado pueden controlar una infestación activa. Para esto asperjamos hojas de litchi con erineo que fueron puestas sobre plantas sanas que luego fueron monitoreadas para el desarrollo de síntomas. Un mes después de la exposición a las hojas tratadas las plantas fueron asperjadas con los mismos tratamientos asperjados en las hojas y monitoreadas para la aparición de síntomas en tejidos jóvenes. Ninguno de los tratamientos resultó en control los ácaros dentro del erineo o en protección de tejidos jóvenes. Los métodos descritos es este estudio pueden utilizarse para realizar evaluaciones más detalladas de otros acaricidas que son requeridos urgentemente para controlar al ácaro erinoso del litchi en Florida.

Palabras Clave: acaricida; tensoactivo organosiliconado; *Aceria litchii*; erineo; cuarentena

The lychee erinose mite, *Aceria litchii* (Keifer) (Acari: Eriophyidae) is an important pest of lychee (*Litchi chinensis* Sonn.; Sapindaceae). This eriophyid mite has been reported in Hawaii (Keifer 1943), Pakistan (Alam & Wadud 1963), China and Taiwan (Huang 1967), Thailand (Keifer & Knorr 1978), India (Prasad & Singh 1981), Australia (Pinese 1981), Bangladesh (Haque et al. 1998), Brazil (Raga et al. 2010; Fornazier et al. 2014), and recently in Florida (Carrillo et al. 2020). *Aceria litchii* feeds on leaf epidermal cells and causes an open gall formation, also known as erinea (Nishida & Holdaway 1955). An erineum is a hyperplasia of plant trichomes induced by the feeding of mites (Royalty

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& Perring 1996; Karioti et al. 2011) and is used as a sheltered habitat for the mites to feed and develop (Jeppson et al. 1975; Sabelis & Bruin 1996). Young new flush is the most susceptible part of the plant; however, the mites can also attack stems, flowers, panicles, and the fruit (Alam & Wadud 1963; Azevedo et al. 2013). *Aceria litchii* infestations can cause up to an 80% decrease in lychee fruit production (Prasad & Singh 1981; Navia et al. 2013).

Management of eriophyid mites is heavily reliant on chemical control. Although chemical control can suppress mite populations, it usually does not eradicate mites from the crop. This is especially true for gall mites, like *A. litchii*, which are protected within the erinea from contact with acaricides. Furthermore, not all acaricides are effective against eggs, resulting in emergence of new individuals after treatment application. Therefore, the timing of application and duration of residual activity are critical parameters to consider when acaricides are used (Childers et al. 1996; Van Leeuwen et al. 2010). Chemical treatments against *A. litchii* aim to protect the new flush. In Queensland, Australia, 3 applications of dimethoate or wettable sulfur at 2 to 3 wk intervals protected the new flush from mite infestation (Waite & Hwang 2002; Waite 2005). Similarly, in Hawaii, Nishida and Holdway (1955) reported that 5 applications with wettable sulfur at 5.6 kg per ha at monthly intervals were sufficient to manage *A. litchii* infestations. In India, dicofol provided the best control of *A. litchii*, whereas in Thailand Schulte et al. (2007) reported that 2 applications of spiromesifen at 0.144 g per L achieved complete elimination of the mite population. The acaricides that have been used in China are dichlorvos, dimethoate, dicofol, chlorpyrifos, and isocarbophos (Waite 2005). None of these reports, however, provided specific efficacy data on the different acaricides due to difficulties in estimating mite populations. Azevedo et al. (2013) developed a laboratory method to maintain live *A. litchii* outside the erinea for 72 h. Using this method, they evaluated the efficacy of acaricides in direct contact with exposed *A. litchii*. Abamectin alone or in combination with a mineral oil, sulfur, hexythiazox, and fenpyroximate caused 96.5 to 100% mortality to *A. litchii*, but also to its natural enemies (Azevedo et al. 2013). However, it remains unknown whether these acaricides can effectively control *A. litchii* inside the erinea and protect the new flush.

Until recently, only 2 acaricides were registered for use on lychee in Florida, bifenazate and abamectin. Bifenazate is an acaricide used against many species of spider mites (Acari: Tetranychidae), but it is not active on gall (Acari: Eriophyidae), broad (Acari: Tarsonemidae), or flat (Acari: Tenuipalpidae) mites (Cloyd 2004). Abamectin is used for a broader spectrum of mite species, including multiple Eriophyidae species, such as the tomato russet mite (*Aculops lycopersici* [Massee]) and the citrus bud mite (*Aceria sheldoni* [Ewing]). The residual activity of abamectin can increase when combined with a horticultural oil or a surfactant. These products assist with translaminar movement and protect the active ingredient from photodegradation (Gent et al. 2003; Khan et al. 2007; Somervaille et al. 2018). In this study we evaluated the ability of abamectin to control *A. litchii* inside erinea and protect new flush with and without a surfactant.

Materials and Methods

ACERIA LITCHII REARING

Seeds were collected from non-infested 'Mauritius' lychee trees grown at the University of Florida, Tropical Research and Education Center in Homestead, Florida, USA. Lychee seedlings were grown in 3.7 L containers filled with ProMix (ProMix BX Mycorhizae, Denver, Colorado, USA) and kept in a room at 25 ± 2 °C, 50% RH, and a 12:12 h (L:D) photoperiod in the arthropod biocontainment facility at the Tropical Research and Education Center. The plants were watered twice per wk and fertilized every 15 d with 24-8-16 (N-P-K) (Miracle-Gro®, The Scotts Company, Marysville, Ohio, USA) and 138 chelated EDDHA iron (Sequestrene®, Syngenta, Wilmington, Delaware, USA). *Aceria litchii*-infested branches of the lychee varieties 'Hak Ip,' 'Mauritius,' and 'O-Hia' were collected from the FruitScapes Nursery (Bokeelia, Florida, USA; 26.38°N 82.07°W) and introduced into the biocontainment facility under the Florida Department of Agriculture and Consumer Services, Division of Plant Industry permit 2018-029. *Aceria litchii*-infested leaflets were detached from the branches and placed on 4 mo old pest-free lychee seedlings showing new flush. Erinea occurrence was assessed by the change of color and trichome density (Carrillo et al. 2020).

DETERMINATION OF TIME NEEDED FOR *ACERIA LITCHII* POPULATION TO LEAVE THE ERINEA

To determine the length of time needed for *A. litchii* mites to exit erinea removed from host plants, 20 *A. litchii*-infested leaflets were chosen randomly and placed individually on a black plastic sheet (11 × 6 cm). It is known that when leaflets start to dry out, mites will leave the erinea (Waite 2005; Azevedo et al. 2013). Because mites were able to walk on the plastic surface, double-sided tape (1.25 cm wide) (Scotch®, 3M, Miami, Florida, USA) was attached to the edges of the sheet to prevent mites from escaping. Every 24 h, the plastic sheets were replaced, and a sub-sample of mites was counted by randomly selecting 3 squares of 1 cm^2. This process was repeated until no mites were found on the plastic sheets.

EVALUATION OF ABAMECTIN AND ORGANOSILICONE SURFACTANT

Leaflets with erinea were inspected for live mites under a stereoscope (Nikon® SMZ1270, Nikon Instruments Inc., Melville, New York, USA) with 400× magnification. Fifty leaflets (10 per treatment) with confirmed presence of live mites were selected randomly to start the experiment. The petioles were inserted individually through a hole in a 1.5 mL Eppendorf® tube (Eppendorf, Enfield, Connecticut, USA) filled with nutrient solution (10N-5P-14K) (MaxiGro®, General Hydroponics Inc., Sebastopol, California, USA) to preserve the plant material during treatment application. The leaflets were affixed to the tube using Parafilm® (Bemis Company, Inc., Neenah, Wisconsin, USA). Individual leaflets were sprayed (Central Pneumatic®, model 93506, Harbor Freight Tools, Taiwan) to run-off with 1 of the following treatments: abamectin 8.0% (Agri-Mek®SC, Syngenta, Wilmington, Delaware, USA) at a concentration of 0.31 L per ha; organosilicone surfactant (DyneAmic®, Helena Chemical Company, Collierville, Tennessee, USA) at a concentration of 5.8 L per ha; a combination of abamectin and organosilicone surfactant at the same concentrations; and water (mechanical control) or non-sprayed (negative control). After treatment application the leaflets were allowed to dry, detached from the Eppendorf® tubes, and placed individually on young, uninfested lychee seedlings. The leaflets remained on the lychee plants for 7 d (based on the results of time needed for *A. litchii* to leave erinea, see previous section), after which they were removed. The lychee plants were kept in a room at 25 ± 2 °C, 50% RH, and a 12:12 h (L:D) photoperiod, and monitored daily for erinea formation for 1 mo. Plants were not in contact with each other, and a water barrier was added below each pot to prevent mite movement from plant to plant.

One mo after placing treated leaflets on the uninfested plants and before developing the new flush, the same treatment was applied to the whole plants. Subsequently, plants were monitored visually for erinea development on the new flush for an additional mo. Non-sprayed plants (negative control) also were monitored for the same duration. Based on the presence of erinea on the new flush, the ability of each product to protect the new flush from a potential infestation was assessed.

STATISTICAL ANALYSIS

To test for differences in the number of lychee plants that developed erinea after exposure to *A. litchii*-infested leaflets, we used a time-to-event analysis (a parametric model with exponential error distribution, package "survival") (Therneau & Foundation 1999). Censoring was applied to lychee plants that did not develop erinea after 1 mo. Treatment was used as a factor and contrasts among treatments were assessed with the estimated marginal means method of the package "emmeans" with a Tukey adjustment of the probabilities ($P < 0.05$). The same type of analysis was used to determine differences in the number of plants that developed erinea on the new flush after treatment application to the whole plants. All analyses were done in R version 3.6.3 (R Development Core Team 2020).

Results

DETERMINATION OF TIME NEEDED FOR *ACERIA LITCHII* POPULATION TO LEAVE THE ERINEA

Within the first 24 h, 97.2% of the *A. litchii* population had left the desiccating erinea and by d 7 there were no mites visible on the plastic sheet (Table 1).

EVALUATION OF ABAMECTIN AND ORGANOSILICONE SURFACTANT

The treatment application to the leaflets had a significant effect on the number of lychee plants that developed erinea (parametric model: deviance = 12.47; df = 4; P = 0.01). All lychee plants receiving non-sprayed or water-sprayed leaflets developed erinea within the first mo (Fig. 1; *t*-ratio = −0.18; df = 45; P = 0.99). Fewer lychee plants developed erinea when they received a leaflet sprayed with abamectin as compared to a leaflet sprayed with water (Fig. 1; *t*-ratio = 2.8; df = 45; P = 0.05). The number of lychee plants receiving non-sprayed leaflets was not significantly different from those receiving leaflets sprayed with abamectin (Fig. 1; *t*-ratio = 2.63; df = 45; P = 0.08). The number of plants receiving leaflets treated with organosilicone surfactant alone or the combination with abamectin did not differ significantly from the number of plants receiving leaflets sprayed with abamectin (Fig. 1; organosilicone surfactant: *t*-ratio = 1.36; df = 45; P = 0.66; organosilicone surfactant + abamectin: *t*-ratio = 0.6; df = 45; P = 0.98).

There was no significant effect of treatment on the number of plants that developed erinea on the new flush (parametric model: deviance = 1.18; df = 4; P = 0.88), indicating that the proportion of plants developing erinea on the new flush was similar for the controls and the treated plants. Of the lychee plants sprayed with abamectin, 70% developed erinea on the new flush within 1 mo, whereas 90% of the plants that were non-sprayed or sprayed with water developed erinea on the new flush within the same time period (Fig. 2).

Discussion

None of the evaluated treatments controlled the mites inside the erinea and protected the new flush. Within the first 15 d of the experiment more than half of the test plants in all treatments had developed erinea (Fig. 1). Lychee plants that received leaflets sprayed with abamectin developed erinea later in comparison with the other treatments (Fig. 1), but the abamectin application to the entire lychee plant could not prevent the mites from infesting the new flush. These results indicate that abamectin alone or in combination with the organosilicone surfactant is not effective for control of mite populations established inside the erinea. Three possible explanations are that (1) the acaricide does not come in contact with the mites at the bottom of the erinea where most of them are located, (2) the acaricide is not efficacious against mite eggs, or (3) the acaricide has a short duration of residual activity and thus the emerging individuals are not controlled.

Abamectin previously was found effective against *A. litchii* by Azevedo et al. (2013). The authors, however, monitored mites that were extracted from sprayed erinea. These results showed that abamectin had a residual activity for 48 h, but it was unclear whether the acaricide can penetrate and kill the mites inside the erinea. Mites could have been in contact with abamectin directly with spraying or while exiting by crawling onto the sprayed trichomes. Horticultural oils or surfactants assist with the translaminar movement of acaricides like abamectin and protect the active ingredient from photodegradation (Gent et al. 2003; Khan et al. 2007; Somervaille et al. 2018). In our experiments, we did not observe an enhanced effect of abamectin when combined with the organosilicone surfactant.

Spraying with products such as sulfur or paraffinic oils may offer better results. Sulfur applications, especially when they are repeated, have given satisfactory results in controlling the *A. litchii* in the field (Nishida & Holdway 1955; Waite & Hwang 2002). Azevedo et al. (2013) showed that sulfur could cause 92.5% mortality within 12 h when applied directly to exposed mites. Recently the US Environmental Protection Agency made available a Special Local Needs label (EPA Registration: 70506-187) for the use of sulfur in lychee. This label has been approved by the Florida Department of Agriculture and Consumer Services, Division of Plant Industry. Although only 1 formulation of sulfur (Microthiol Disperss®, UPL, King of Prussia, Pennsylvania, USA) is available currently, it is necessary to experimentally demonstrate the ability of sulfur to protect the new flush or control an existing *A. litchii* infestation. Perhaps prophylactic applications before the mite appears in the crop can protect the new flush; however, this remains to be tested. Paraffinic oils have been successfully proven to disinfest *A. litchii* of lychee fruit (Revynthi et al. 2020). Additional experiments to test the efficacy of such oils against *A. litchii* may reveal a useful alternative to conventional acaricides.

Aceria litchii is being managed in different countries by adopting cultural practices which include pruning and burning infected twigs (Fornazier et al. 2014; Castro et al. 2018). Recommended practices

Table 1. *Aceria litchii* population exiting the erinea of desiccating 'Maritius' lychee leaflets within 7 d. Average percentage (± SE) of N = 20 infested lychee leaflets.

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
97.2% (± 0.03%)	0.6% (± 0.02%)	0.8% (± 0.02%)	0.9% (± 0.02%)	0.3% (± 0.01%)	0.2% (± 0.01%)	0% (± 0%)

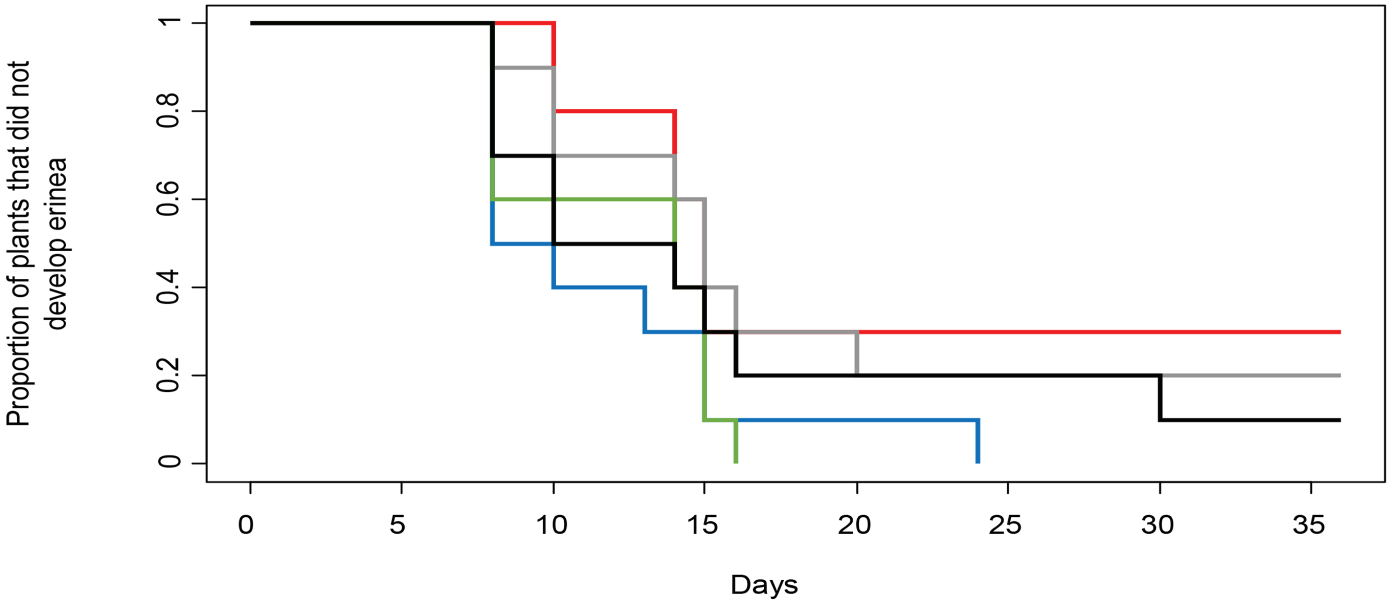

Fig. 1. Proportion of lychee plants that did not develop erinea after *Aceria litchii* infested leaflets were placed on them. Leaflets were sprayed with abamectin (red), organosilicone surfactant (black), a combination of abamectin and organosilicone surfactant (grey), water (blue), or non-sprayed (green). Shown are average proportions of lychee plants through time. *N* = 10 for each treatment.

include strict pruning followed by acaricide application (Castro et al. 2018). Lychee varieties, such as 'Deshi' and 'Kasba' in India (Ranjan & Kumar 2018), and 'Mauritius' (Arantes et al. 2017) and 'Americana' (Amaral et al. 2020) in Brazil, have been found to be more tolerant to *A. litchii*. These findings indicate that plant breeding might provide another solution for management of this pest. Moreover, several natural enemies were associated with *A. litchii*, including mites and insects (Waite & Gerson 1994; Picoli et al. 2010; Navia et al. 2013; Azevedo et al. 2014). Adopting solely chemical control as a management method will have a detrimental impact on natural enemies that can control *A. litchii* populations (Azevedo et al. 2013). Working in a containment facility with a specialist fruit crop pest is challenging. We show how an *A. litchii* colony can be established successfully using lychee seedlings. To the best of our knowledge this is the first report of a successful laboratory *A. litchii* colony. All previous studies have been conducted either in the field or have used field populations in laboratory experiments.

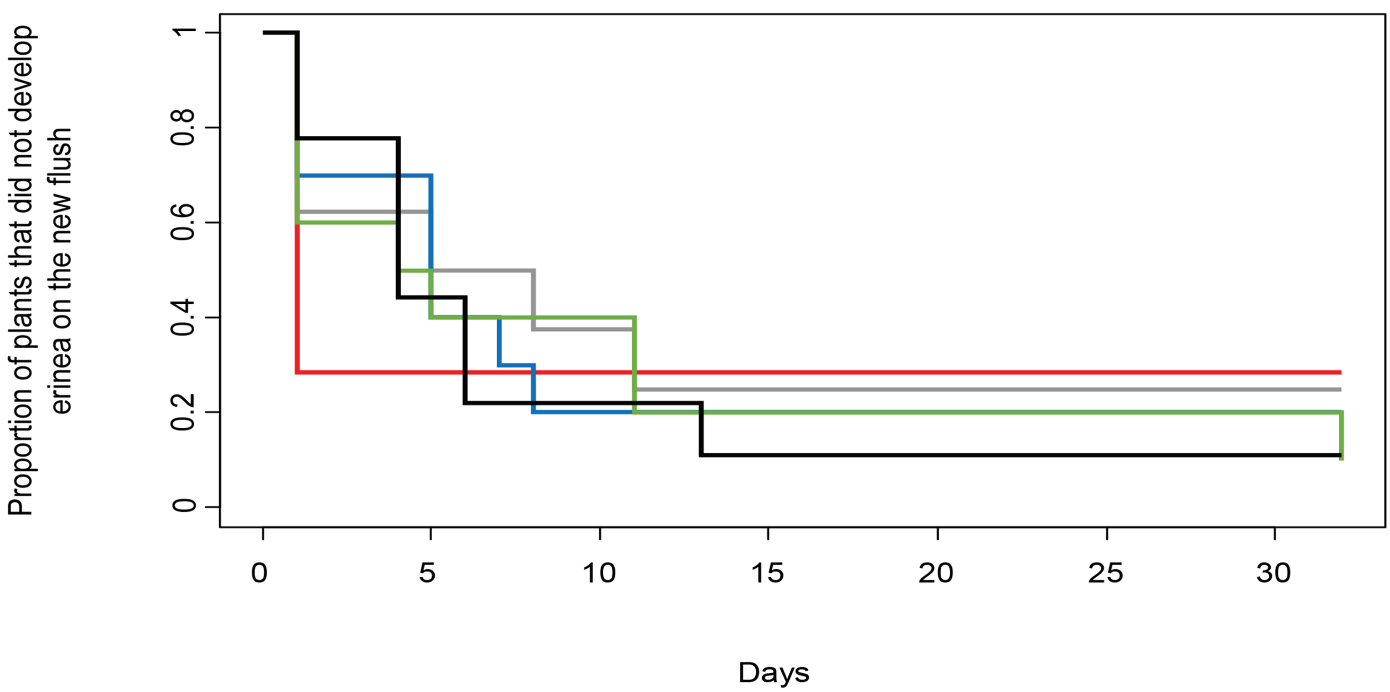

Fig. 2. Proportion of lychee plants that did not develop erinea on the new flush after being sprayed with the treatment that was previously applied to the received leaflet. Lychee plants were sprayed with abamectin (red, *N* = 7), organosilicone surfactant (black, *N* = 9), combination of abamectin and organosilicone surfactant (grey, *N* = 8), water (blue, *N* = 10) or non-sprayed (green, *N* = 10). Shown are average proportions of lychee plants through time.

Our study demonstrates that with the sole use of abamectin it is not possible to eliminate an established *A. litchii* population or to protect the new flush. Therefore, it is necessary to study and implement other chemical alternatives to a balanced integrated pest management program.

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