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Repellency of kaolin particle film to common pistachio psyllid, *Agonoscena pistaciae* under field conditions

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Abstract

The common pistachio psyllid, *Agonoscena pistaciae* Burckhardt et Lauterer (Hemiptera Psyllidae) is the key pest of pistachio trees in Iran. Current management practices of this pest in pistachio orchards are based on insecticide applications, with as many as 6 applications during the growing season. In order to reduce reliance on insecticides, an alternative may be the application of alternative compounds which alter the attractiveness of pistachio trees for common pistachio psyllid feeding and oviposition. In this study, the effect of different concentrations of kaolin particle film (1, 2.5, 3.5, 5, 6 and 7.5%) on oviposition rate and nymph population density was investigated utilizing a randomized complete block design under field conditions in 2013 and 2014. Sampling was carried out 2, 7, 14, 21 and 28 days after treatment. Results indicated 91% to 100% reduction in oviposition at ≥ 5% concentrations. Generally, no eggs were found on the leaflets of pistachio trees at all sampling dates with 7.5% concentration, and at the first three sampling dates for 6% concentration, in both 2013 and 2014. Significant differences between 5% concentration and 6 and 7.5% concentrations were noted only 28 days after treatment. The highest and lowest reduction of nymph population density was observed with 7.5% concentration (> 90%) and with 1% concentration (> 20%), respectively. At the higher concentrations tested, kaolin particle film significantly reduced common pistachio psyllid oviposition. Utilization of kaolin particle film may be an effective strategy in integrated management of common pistachio psyllid.

Key words: *Agonoscena pistaciae*, population density, kaolin, particle film technology, repellency, physical barrier.

Introduction

The pistachio, *Pistacia vera* L. (Sapindales Anacardiaceae), is one of the most important economic crops in Iran (Mehrnejad, 2010). The common pistachio psyllid (CPP), *Agonoscena pistaciae* Burckhardt et Lauterer (Hemiptera Psyllidae), is one of the most detrimental pests to pistachio trees (Rouhani *et al*., 2012). The presence of high population densities of psyllid nymphs and adults causes severe problems in kernel development and leads to subsequent bud drop and defoliation, resulting in significant economic losses. For this reason, psyllid infestations have received particular attention from pistachio-growers, who insist on spraying to reduce the psyllid damage (Mehrnejad, 2001). Current management of this pest relies primarily on application of insecticides (as many as 6 applications) in individual pistachio orchards during the growing season in Rafsanjan, Iran (Samih *et al*., 2005). Unexpected side effects of pesticide applications, such as pest resistance, pest resurgence and outbreaks of secondary pests may occur when overuse of insecticides is employed in crops over prolonged periods. These harmful side effects show the need for alternative methods of control that do not rely on insecticides alone (Azimizadeh *et al*., 2012). In many countries, there is an increasing effort to develop novel approaches for management of invertebrate crop pests. The physical environment of the pest can be modified in such a way that the insect no longer poses a threat to the agricultural crops (Vincent *et al*., 2003). Therefore, incorporation of methods which reduce the attractiveness of pistachio trees to CPP may serve to complement and reduce reliance on insecticides. A decrease in attractiveness can result from a negative tactile response or through interference with the host plant physiology (change in olfactory and/or visual cues) (Peng *et al*., 2011). The use of particle film technologies has recently been introduced as a novel approach to suppress arthropod pests of crops (Glenn *et al*., 1999). Particle film technology synthesizes knowledge of mineral technology, insect behaviour and light physics in order to control pests (Glenn and Puterka, 2005). Kaolin, the main component of these particle films, is a white, non-porous, non-swelling and non-abrasive aluminosilicate mineral ($Al_4Si_4O_{10}[OH]_8$) that easily disperses in water and is chemically inert over a wide pH range (Glenn *et al*., 1999). Powdered suspensions of processed kaolin cause a barrier and repellent effect against insects (Glenn *et al*., 1999; Puterka *et al*., 2000). Particle films are effective against many key orders of arthropod pests affecting crops, including homopterans, coleopterans, lepidopterans, dipterans and rust mites (Glenn and Puterka, 2005). Such technology has effectively suppressed several pests such as *Cacopsylla pyricola* Foerster (Hemiptera Psyllidae) on apple and pear (Glenn *et al*., 1999; Pasqualini *et al*., 2002; Puterka *et al*., 2005), *Diaphorina citri* Kuwayama on citrus (Hall *et al*., 2007; Puterka *et al*., 2005) and *Bactrocera oleae* (Rossi) (Saour and Makee, 2003; Pascual *et al*., 2010). Kaolin-based particle films create a white powdery film after spraying, which serves as a physical barrier repelling arthropods and/or suppressing infestations by making the plant visually or tactily unrecognizable as a host. Furthermore, it hampers insect movement, feeding and other physical activities (Glenn *et al*., 1999; Puterka *et al*., 2000; Showler, 2002; Wyss and Daniel, 2004). Application of kaolin particle film provides many benefits; it is unlikely that pests will develop resistance to it (Liu and Trumble, 2004), it has no phytotoxic effects, it lasts longer on the plants than most insecticides (Sugar *et al*., 2005) and it is non-toxic to humans and relatively safe to natural enemies (Friedrich *et al*., 2003; Delate and Friedrich, 2004). Kaolin particle film could prevent incipient radiation, preserve heat, regulate temperature and enhance maturity in plants (Wisniewski *et al*., 2002; Wand *et al*., 2006). Additionally, it forms a suspension in water, can be easily applied using conventional spray equipment and may eventually reduce the number of applications of conventional insecticides. A complete and uniform film coating on the plant surface is essential for kaolin particle film to be effective (Glenn *et al*., 1999; Larentzaki *et al*., 2008). Although kaolin particle film has previously been evaluated for management of pistachio psyllid *Agonoscena targionii* (Lichtenstein), the effect different concentrations of kaolin on population density reduction (eggs and nymph) has not been evaluated. Therefore, the aims of the present study were: (1) to confirm the repellent effect of kaolin against CPP and (2) to test different concentrations of kaolin in order to determine the optimum concentration for control of CPP under field conditions on pistachio trees.

Materials and methods

Kaolin particle film

The kaolin particle film (Sepidan®) used in this study was a hydrophilic film (WP; a.i. 95%) that was provided from Kimia Sabzavar Company, Iran.

Study site

The trial was conducted in a 20-year-old ‘Akbari’ pistachio orchard with high density of CPP in Rafsanjan, Iran during summer 2013-2014. The experimental orchard was a moderate density plantation (1000 trees/ha). The orchard consisted of 30 rows with 34 trees per row. The trees (2.5 m height) were spaced 4 m apart. The trial was arranged in a completely randomized block design with four replications (each plot consisted of 228 trees) and seven treatments including (kaolin 1, 2.5, 3.5, 5, 6 and 7.5%), along with an untreated control (water treatment) under field conditions. Kaolin was applied to trees by spraying them to runoff with a small motorized sprayer equipped with an agitator. The trees were treated once per year.

Sampling of eggs

For sampling of each treatment, four trees in the centre of rows were selected randomly; from each tree, five leaves from each side (totally, 20 leaves) were picked randomly. The numbers of eggs laid on upper and lower sides of each apical leaflet in each plot were counted under a stereomicroscope. Sampling was undertaken 2, 7, 14, 21 and 28 days after kaolin application. Oviposition deterrent percentage (O.D. %) of the kaolin treatment was calculated according to the following formula (Lundgren, 1975):

$$O.D.\ \% = (B - A) / (A + B) \times 100$$

where, A = mean number of eggs laid on the treatment, B = mean number of eggs laid on the control.

Sampling of nymph population

The sampling was carried out a day before treatment and 2, 7, 14, 21 and 28 days post-treatment. Sampling was undertaken in the same way as egg sampling. The nymphs of CPP on upper and lower sides of each apical leaflet were counted and recorded. Mean mortality was calculated and data corrected by Henderson-Tilton formula as below:

$$\text{Efficacy}\ \% = [1 - (Ta / Ca)\ (Cb / Tb)] \times 100$$

where, Ta = mean number of living pests in treated plot after treatment, Ca = mean number of living pests in control plot after treatment, Cb = mean number of living pests in control plot before treatment, Tb = mean number of living pests in treated plot before treatment (Henderson and Tilton, 1955).

Statistical analysis

All data (%) were arcsine square-root transformed before statistical analysis. Data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) (SPSS 16, Inc). The means were separated by Tukey’s test ($P < 0.05$).

Results

Oviposition reduction

2 0 1 3

There were significant differences in percentage of oviposition reduction among different kaolin treatments 2 days ($F = 304.10$; df = 5, 3; $P = 0.0001$), 7 days ($F = 905.41$; df = 5, 3; $P = 0.0001$), 14 days ($F = 320.41$; df = 5, 3; $P = 0.0001$), 21 days ($F = 296.04$; df = 5, 3; $P = 0.0001$) and 28 days ($F = 767.277$; df = 5, 3; $P = 0.0001$) after treatment ($P < 0.05$). The least and greatest repellency were found in 1% (47.25%) and 7.5% (100%) treatments, respectively 2 days after treatment (figure 1). Generally, three lower concentrations (1, 2.5 and 3.5%) showed significant differences at all sampling dates (figure 1). There were no significant differ-

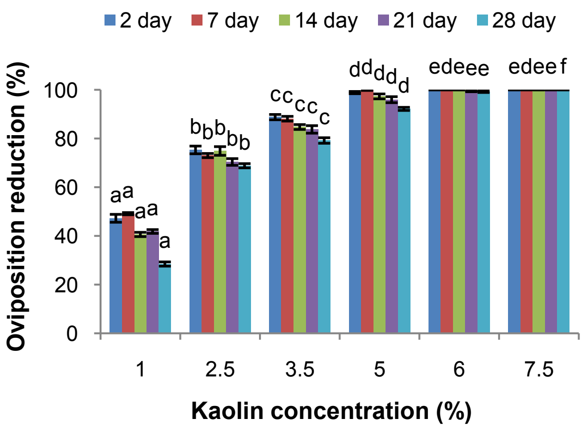

Figure 1. Means (± SE) percent reduction of CPP eggs at different times after treatment in 2013. The means, in the day after treatment, followed by the same letters do not differ significantly ($p < 0.05$, Tukey’s test). (In colour at www.bulletinofinsectology.org)

ences between 6 and 7.5% concentrations except 28 days after treatment (figure 1). As shown, no eggs were observed in 6% concentration at the first three dates. Therefore, 21 days after treatment for the first time, eggs were observed in 6% concentration (99.47% oviposition reduction). In contrast, at all sampling dates, no eggs were found in 7.5% concentration. Finally, 28 days after treatment, the lowest and highest reductions in oviposition were recorded 28.47 and 100% in 1 and 7.5% concentrations, respectively (figure 1).

2 0 1 4
Results indicated that there were significant differences in percentage of oviposition reduction among different kaolin treatments 2 d ($F = 174.61$; $df = 5, 3$; $P = 0.0001$), 7 d ($F = 572.34$; $df = 5, 3$; $P = 0.0001$), 14 d ($F = 551.63$; $df = 5, 3$; $P = 0.0001$), 21 d ($F = 507.77$; $df = 5, 3$; $P = 0.0001$) and 28 d ($F = 357.55$; $df = 5, 3$; $P = 0.0001$) after treatment ($P < 0.05$). The lowest oviposition reduction was recorded in 1% concentration (41.75%), while no eggs were found (100% oviposition reduction) in 5, 6 and 7.5% concentrations 2 days after application (figure 2). Two weeks after kaolin application, for the first time, eggs were observed in 5% concentration but showed no significant differences with 6 and 7.5% concentrations (figure 2). The third week, 6% concentration (99.23%) showed no significant differences with 5 (96.23%) and 7.5% (100%) concentrations (figure 2). At the 28 days sampling date in 7.5% concentration there were no eggs; the lowest oviposition reduction (23.73%) was recorded for 1% concentration. Also, only on 28 days sampling date, 5% concentration (91.07%) showed significant differences between concentrations 6 (98.27%) and 7.5% (100%) (figure 2). A decreasing trend of repellency was observed for all the treatments with the passing of time except for 7.5% and ≈ 6% concentrations (figure 2).

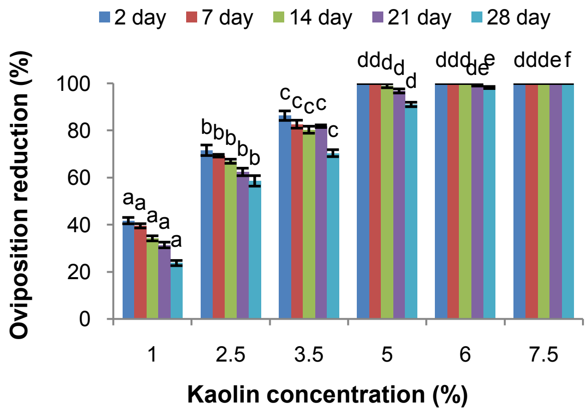

Figure 2. Means (± SE) percent reduction of CPP eggs at different times after treatment in 2014. The means, in the day after treatment, followed by the same letters do not differ significantly ($p < 0.05$, Tukey's test).
(In colour at www.bulletinofinsectology.org)

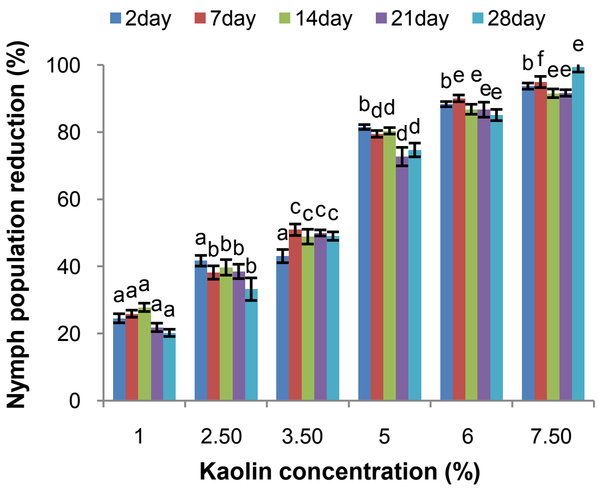

Figure 3. Means (± SE) percent reduction of CPP nymphs at different times after treatment in 2013. The means, in the day after treatment, followed by the same letters do not differ significantly ($p < 0.05$, Tukey's test).
(In colour at www.bulletinofinsectology.org)

Reduction of nymph population density

2 0 1 3
There were significant differences in nymph density among different kaolin treatments 2 days ($F = 647.285$; $df = 5, 3$; $P = 0.0001$), 7 days ($F = 411.45$; $df = 5, 3$; $P = 0.0001$), 14 days ($F = 292.12$; $df = 5, 3$; $P = 0.0001$), 21 days ($F = 255.6$; $df = 5, 3$; $P = 0.0001$) and 28 days ($F = 206.17$; $df = 5, 3$; $P = 0.0001$) after treatment ($P < 0.05$). 2 days after treatment the greatest and least reduction of nymph density were found in 7.5% (93.7%) and 1% (24.54%) concentrations, respectively (figure 3). On this day, 1, 2.5 and 3.5% concentrations showed no significant differences. Also there were no significant differences among 5, 6 and 7.5% concentrations (figure 3). There were significant differences among all concentrations 7 days post-treatment (figure 3). Also, there were no significant differences between 6 and 7.5% concentrations 14, 21 and 28 days after application (figure 3). Finally, at the 28 days sampling date, the highest and lowest nymph density reduction were recorded in 7.5% (92.35%) and 1% (20.2%) concentrations, respectively (figure 3).

2 0 1 4
Results indicated that there were significant differences in nymph density among different kaolin treatments 2 days ($F = 221.22$; $df = 5, 3$; $P = 0.0001$), 7 days ($F = 501.82$; $df = 5, 3$; $P = 0.0001$), 14 days ($F = 272.05$; $df = 5, 3$; $P = 0.0001$), 21 days ($F = 481.47$; $df = 5, 3$; $P = 0.0001$) and 28 days ($F = 881.621$; $df = 5, 3$; $P = 0.0001$) after treatment ($P < 0.05$). There were no significant differences between 2.5 and 3.5% concentrations and also between 5 and 6% concentrations at 2 days after treatment (figure 4). At the 2 days sampling date, the highest nymph density reduction was recorded in 7.5% concentration (92.05%), which at 7 days after treatment showed a slight increase (94.2%). Also at 2 days, lowest nymph density reduction was found in 1% concentration (23.35%), which at 7 days after kaolin

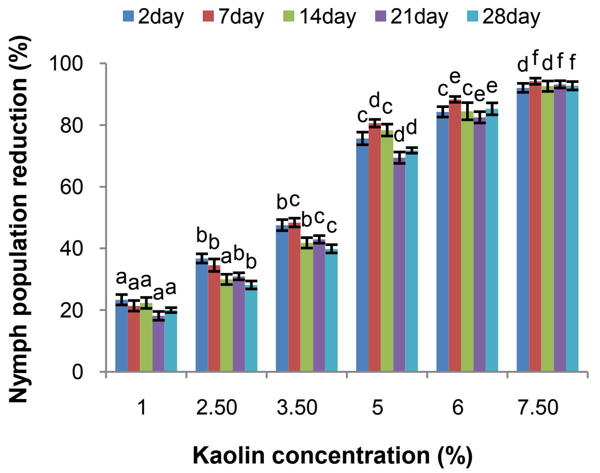

Figure 4. Means (± SE) percent reduction of CPP nymphs at different times after treatment in 2014. The means, in the day after treatment, followed by the same letters do not differ significantly ($p < 0.05$, Tukey's test).
(In colour at www.bulletinofinsectology.org)

application showed a further decrease (21.38%) (figure 4). At the 14 day sampling date, 5% concentration (78.36%) was not significantly different from 6% concentration (84.48%). Also, there were no significant differences among two lower concentrations 1 (22.31%) and 2.5% (29.94%) on day 14 (figure 4). There were significant differences among the all concentrations at 2, 21 and 28 days after treatment. Finally, at the 28 days sampling date, the highest and lowest nymph density reduction was recorded at 92.73% and 19.99% in 1 and 7.5% concentrations, respectively (figure 4).

Discussion

In the present study, not only were no eggs on upper and lower side of leaflets of pistachio trees at all sampling dates in 7.5% concentration but these results were also observed at the first three sampling dates in 6% concentration in 2013 and 2014. Therefore, 7.5% concentration showed the strongest repellency to females and deterred oviposition at different days after treatment. Repellency to female oviposition was decreased at 1, 2.5 and 3.5% concentrations especially 28 days after kaolin application while 6 and 7.5 and ≈ 5% concentrations remained highly effective four weeks after application. On the other hand, a trend towards a decreasing repellency effect for all treatments was observed with the passage of time, except in 7.5 and ≈ 6% concentrations (figures 1 and 2); in 5% concentration, only a slight decrease was observed after 28 days. Generally, kaolin treatment at 5, 6 and 7.5% concentrations suppressed the CPP infestations, while 1 and 2.5% concentrations failed to do so. The concentration 3.5% did not provide adequate protection against CPP damage. The reduction of oviposition due to kaolin could be attributed to the repellency effect of kaolin. Our study clearly demonstrated that kaolin protects pistachio trees against infestations of CPP. Results of present study are consistent with results of Erler and Cetin (2007) that reported oviposition deterrency of a 1% concentration kaolin particle film on pear psylla at levels of 50.5 and 66.9% after four weeks, in 2004 and 2005, respectively. Also, Pasqualini *et al.* (2002) reported kaolin has a very good efficacy (99-100% reduction in mean egg numbers) in comparison to mineral oil and untreated control on *Cacopsylla pyri* (L.). As well as, Hall *et al.* (2007) showed that the numbers of eggs of *D. citri* on kaolin-treated citrus trees (3%) per flush shoot was reduced by 85%. When kaolin particles cling to the body of the insect pest, the insects struggle on treated surfaces, thus leading to feeding and oviposition reduction (Glenn and Puterka, 2005). Moreover, Peng *et al.* (2011) reported potato psyllid adults, *Bactericera cockerelli* (Sulc) could land on kaolin-treated plants when no choice was given, but fewer eggs were laid. When given a choice, psyllids avoided treated plants under laboratory and field conditions. Saour (2005) reported that kaolin-sprayed pistachio trees throughout two years of trials were healthier and more vigorous than pistachio trees treated with chemical insecticide for control of *A. targionii*. Although the modes of action of kaolin particles on insects and mites were not fully understood, it appears that these effects may be attributed to colour (white) treated surface, which repels the insects from landing (Liang and Liu, 2002) and disrupts insect feeding and oviposition (Liang and Liu, 2002; Liu and Trumble, 2004). In addition, Glenn *et al.* (1999) reported that pear psylla oviposition could also be severely reduced by the attachment of kaolin particles to adult's body as they crawled upon the particle film. These reports showed that adults were distracted, either trying to remove the particles from their body parts or remaining motionless compared to untreated adults (feed and lay eggs actively). Due to the effect of kaolin particles on the feeding behaviour of pear psylla adults, Glenn *et al.* (1999) suggested that the behaviour of the adults was disrupted by the clinging particles to their body; consequently, they were unable to feed and eventually starved after 3 days. Nymph population density in the present study was reduced in different concentrations of kaolin, especially in 5, 6 and 7.5% concentrations over the two study years. The highest and lowest reduction in population density was observed in 7.5% and 1% concentrations, respectively. Our findings are supported by Pasqualini *et al.* (2002) which showed that two applications of kaolin in February and March caused a 99–100% reduction of *C. pyri* nymphs. In this study, kaolin-treated plots showed lower levels of nymph instars depending on concentration utilized and especially in 5, 6 and 7.5% concentrations. The present data showed that kaolin particle film was highly effective to control of CPP by preventing egg laying and subsequent build-up of population density. Puterka *et al.* (2005) noted that in kaolin-treated trees (3%), young nymphs of *C. pyricola* were showed moderate levels of early mortality. Hall *et al.* (2007) showed that the numbers of citrus psyllid nymphs per flush shoot were reduced by 78% in kaolin particle film-treated trees. Repellency of kaolin particle film to various insects has been reported in the literature.

Kaolin was successfully used in laboratory and field experiments against the European pear sucker, *C. pyri,* significantly reducing the population density of this polyvoltine species (Daniel *et al*., 2005; Erler and Cetin, 2007). Results of Peng *et al.* (2011) regarding the effect of kaolin on potato psyllid, *B. cockerelli* showed the presence of fewer numbers of adults, eggs and nymphs on kaolin particle film-treated plants than on control plants. As well, results of this study are consistent with finding of other research undertaken on several psyllid species (Puterka *et al*., 2000; Pasqualini *et al*., 2002; Daniel *et al*., 2005; Saour *et al*., 2005). Glenn *et al.* (1999) and Puterka *et al.* (2000) reported oviposition of the winter and summer form of pear psylla was significantly suppressed by the application of kaolin, and suppression of oviposition resulted in significantly fewer nymphs in particle film treatments compared with untreated controls. Due to the mechanism (s) involved in suppressing oviposition, Puterka *et al*. (2000; 2005) suggested that alterations of colour and/or cuticle structure of tree bark by the application of kaolin could be responsible for reduced oviposition of pear psylla. The non-abrasive and soft nature of kaolin indicates that this compound is ineffective as an insecticidal agent (Glenn *et al*., 1999). Therefore, kaolin particle film mode of action is to prevent psyllids from feeding behaviour, as reported with aphids. The early onset of a change in these pests behaviour may be related to altered visual or tactile cues of plant (Glenn *et al*., 1999; Burgel *et al*., 2005).

CPP management in Iran is directed primarily toward reducing the population density prior to the pistachio rapid growth kernel stage using synthetic insecticides. This study suggests that kaolin may offer a suitable alternative to control of this key pest before this critical stage and prevent early season population build-up. Although it is not clear whether kaolin particle film can completely replace chemical insecticides in the management of CPP on pistachio, it may be a useful and less toxic alternative within an integrated pest management strategy. As well, use of botanical oils and essential oils can enhance kaolin efficacy, as previously demonstrated (Liang and Liu, 2002; Reitz *et al*., 2008), where application of kaolin and oils was studied to evaluate the synergist effect of oil on kaolin. Since rainfall levels are very low in pistachio planting areas of Iran, application of kaolin seems to be a suitable strategy for CPP control. Our results, like those reported in other studies, have shown that non-chemical kaolin is an effective insecticide, without side-effects such as phytotoxicity on treated plants. In summary, the present study has clearly indicated the potential of kaolin particle film as a repellent to common pistachio psyllid, *A. pistaciae* and has demonstrated that kaolin particle film might be an economically viable and environmentally sound component of an integrated approach for control of CPP and related pests.

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