Journal of Biological Engineering Research and Review, 2017; 4(2): 31-37
ISSN: 2349-3232 Online
Available online at www.biologicalengineering.in/Archive

Research Article

Attraction and Oviposition Stimulation of Gravid *Aedes* Female Mosquitoes Using Different Colored Earthen Ovitraps in Field Areas

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ARTICLE INFO:

Article History:
Received: 12/06/2017
Revised: 06/12/2017
Accepted: 19/03/2018
Available Online: 22/03/2018

Keywords:
Aedes aegypti; Ae. albopictus; Oviposition; larvae; Color

Citation: Mya MM, Maung KS, Oo NN, Maung YNM, Htun MM. Attraction and Oviposition Stimulation of Gravid Aedes Female Mosquitoes Using Different Colored Earthen Ovitraps in Field Areas. Journal of Biological Engineering Research and Review. 2017, 4(2), 31-37.

Abstract: Wild gravid female *Aedes aegypti and Ae. albopictus* were allowed to lay their eggs in man-made earthen ovitraps with different colors as black, blue, green, orange, red, white, yellow and control (without color). The ovitraps were used indoor and outdoor for oviposition preference from March 2016 to December 2016. Oviposition cycle was studied 14 days/cycle trial in different places for 84 days. The number of larvae and frequency of oviposition were observed. Experiments were conducted to determine egg laying preference for any specific color of the ovitrap. Results showed that white colored bowl in indoor and red colored bowl in outdoor were most preferred by *Ae. aegypti* and black ovitrap was to be most preferred for *Ae. albopictus* in outdoor, although black colored bowls were preferred by both the species in outdoor. Gravid *Ae. aeqypti* laid their eggs in all colored bowls in both indoor and outdoor. For oviposition preference, seven colored earthen pots were used in indoor and outdoor, and the combined result was observed that the maximum number of larvae was found in a red colored bowl followed by black colored bowl containing distilled water. Besides, *Aedes* mosquitoes laid more in the red bowl followed by the Yellow colored bowl; lowest laid frequencies were found in orange bowls. *Ae. albopictus* was laid five times in outdoor in the black bowl within 84 days. In the present studies, *Ae. aegypti* laid the maximum number of eggs in red colored bowls and *Ae. albopictus* laid the maximum number only in black colored bowl in outdoor. The study reveals that the color of the ovitraps plays an essential role in oviposition by attracting the females of *Ae. aegypti* species. The ovitraps of red, yellow, black and white color were found to be appropriate for *Aedes* eggs and larvae collection to control *Aedes* population in field areas.

INTRODUCTION

Mosquitoes are medically important insects and the most widely spread in tropical and subtropical regions in the world. Regarding both morbidity and mortality, dengue is the most common and widespread viral infection to human in the world today [1]. The geographical distribution and spread of incidence as well as the severity of dengue fever (DF) and dengue hemorrhagic fever (DHF) are increasing in the Americas, Eastern Mediterranean, the Caribbean, Southeast Asia, and Western Pacific. Three billion people live in areas where potentially at risk of dengue virus infection and transmission. Annually, it is estimated that globally, there are 20 million cases of dengue infection, resulting in around 24,000 fatalities [2].

In Myanmar, a severe outbreak of DHF occurred for the first time in Yangon in 1970 [3]. This epidemic had an affected mostly school going children groups. Dengue fever (DF) and DHF are increasingly becoming severe public health problems especially among the 5-10 and 11-15 years old age groups and now noted 15 years above, a vast majority of the cases occur in 5-8 years old age group [4,5]. The highest number of cases and deaths recorded were 9149 DHF cases and 55 deaths recorded across Myanmar [6]. More DHF cases predominate during the raining season, especially in July and August. The highest number of cases was recorded in July [3]. Dengue viruses are transmitted to humans through the bite of infected *Aedes* mosquitoes, principally *Aedes aegypti*. *Aedes aegypti* is a tropical and subtropical species of mosquito found around the globe, usually between the latitudes 35^{0}N and 35^{0}S. *Aedes aegypti* is one of the most efficient mosquito's vectors in urban areas and *Ae. albopictus* is a most efficient vector in rural areas. It is highly anthropophilic and thrives close to humans. Dengue outbreaks have also been attributed to *Ae. albopictus*, *Ae. polynesiensis* and several species of the

Aedes scutellaris complex. Each of these species has its particular geographical distribution. So far, they have been less efficient epidemic vectors than *Ae. aegypti* [2]. Both *Ae. aegypti* and *Ae. albopictus* are found in Myanmar, Malaysia, Thailand, Indonesia, etc. through *Ae. aegypti* is not an indigenous species [3,7]. *Aedes aegypti*, is highly adapted household-container breeder [8]. Vision plays a principle role in adult mosquito biology, including location of hosts, food sources, mates, resting sites, and oviposition sites [9]. A large number of studies have examined the visual parameters of shape, size, contrast, light intensity, texture, and color attraction to host-seeking mosquitoes, while few studies have explored that which of these parameters are attractive to gravid adult females [10]. Most oviposition attraction studies seek to uncover specific colors generated from microbial agents that are responsible for attracting gravid females to a potential oviposition site; however, site selection is also dependent on tactile and visual cues with vision possibly as important as olfactory cues in site selection among some mosquito species [11].

Oviposition is an essential component of most mosquito-borne diseases [11]. A tremendous amount has been written concerning the oviposition of mosquitos including *Aedes* species [12,13]. Selection of oviposition sites by gravid female mosquitos is a crucial event for the survival of their eggs, larvae and their species. The presence of aquatic predators of mosquito eggs, larvae, or pupae is a risk that some species take into account before oviposition [14]. A large proportion of the studies have dealt with laboratory and field investigations of mosquito responses to the chemical and physical species of the oviposition site [15,16], but relatively few field studies have been attempted locally. Mosquitoes have preferential oviposition habits. Water collection, human activities, and behavior, planting ornamental plants around houses may potentially encourage the breeding of mosquitoes [17]. Ovitraps are being employed as a sensitive method in detecting the presence of *Ae. aegypti* [18]. They are considered more sensitive than larval surveys where the population density is low and larval surveys are largely unproductive [19-20]. Several improvements and modifications have been made to enhance the efficacy of ovitraps. Size, color, material, paddle, and oviposition attractant have been reported to be the important factors that influence the efficacy of the ovitrap [21]. In some areas, there is no water supply and timely water supply areas due to the shortage of water, the people tend to store water for a longer period in different color containers which support and enhance the mosquito breeding, and their color may play an important role in container choice among gravid females. Despite several research works on mosquitoes in Myanmar, very little is known of the degree to which the visual parameters of color and contrast influence oviposition site selection of either species. More information is needed to be required about their color preferences particularly on DHF vectors *Aedes* mosquitoes. Keeping in view this aspect, the present studies, have been planned and since different colored ovitraps have been considered to study the color preference of the gravid females of *Aedes* mosquitoes for the surveillance of *Aedes* mosquitoes, as an important surveillance tool, in public areas.

MATERIALS AND METHODS

Study area

Study was conducted in No. 2, 4 and 8 quarters of Pakokku Township, Magwe Region. These three quarters are the most significant large population in the Township. Primary schools and monastery schools are available in each quarter. About 5-7 thousand population live in each quarter. There is no government water supply in all quarters. Most people used wells and tube wells water in their place. They store the water in water storage containers for general used. Therefore, *Aedes* mosquito density is high in these areas. In the study areas most of the peoples are farmers, government staff, and teachers.

Study period

Study period was conducted from January 2016 to February 2017 (One-year study).

Study procedure

One set of ovitraps (Oneliter size eight earthen bowls: one bowl without colored for control and seven bowls were painted with seven different colors as yellow, green, red, blue, white, black and orange) was made individual set for six sets. These six sets man-made earthen container traps were filled with equal amounts (500ml) of distilled water, and one set of different colored container traps was placed indoor in the randomly selected house, and one set was placed outside in the garden of the houses. Another two sets (one indoor and one outdoor) were placed in another house which was far away 300 meters from starting place. Another two sets were placed indoor and outdoor as above procedure. All total of six sets (3sets indoor and three sets outdoor) were used for determination of species abundance and color preferences against oviposition preferences of mosquitoes in selected quarters of Pakokku Township. These traps were placed under shade at the 5cm interval between traps. The colors were set alphabetically as follows; black, blue, green, orange, red, white and yellow. The containers were observed daily for 14 days. On each observation day, all the vessels were carefully followed for the occurrence of mosquito larvae.

Sampling Procedure

The content of each container was taken and poured into a clean and white empty bowl. The total number of the larvae were then counted and recorded. After scoring, a pipette was used to transfer all the instar larvae into a separate Petri dish containing some water. All the Petri dishes were carried to the laboratory for the species identification of larvae. The 1^{st} and 2^{nd} instars larvae were reared till they developed into the 3^{rd} and 4^{th} stage instar larvae. At the time larvae were put in 80% ethyl alcohol and allowed to die. Then the death larvae were identified under the magnification of 40x10 by compound Olympus microscope for proper species identification.

Species identification

Aedes species identification was made according to different authors [22,23].

Statistical analysis

Field and laboratory data were recorded in appropriate forms, and statistical analysis was conducted using Microsoft Excel software. Also, comparison of oviposition preference in different colors of positive traps between indoor and outdoor was also used to calculate by Microsoft Excel.

RESULTS

Table 1 shows, oviposition of *Aedes* mosquitoes in a different colored bowl in field areas. Within 84 days trial in indoor, the highest *Aedes* larvae were collected in white coloured bowl followed by Green and Red, Black, and Yellow coloured bowls respectively and lowest larvae were observed in Blue and Orange bowls in indoor. Within 84 days trial in outdoor, the highest *Aedes* larvae were collected in the Red bowl followed by Black, Yellow and Blue bowls respectively. Lowest larvae were collected in the Orange, White and Green bowls respectively. In the comparison, *Aedes* mosquitoes oviposited in all different colored bowls of indoor and outdoor. Although the high numbers of larvae were collected in White, Red, Yellow, Green and Black bowls in indoor. In outdoor, the high numbers of larvae were collected in Red, Black, Yellow, Green, Blue and Orange colored bowls. When added the larvae from indoor and outdoor, the highest number of *Aedes* larvae were collected in Red bowl followed by Black bowl and lowest number was found in the Orange bowl. White, Yellow, Blue and Green bowls were found the moderate amount of larvae, respectively.

Table 1: Comparison of the mean number of immature *Aedes* mosquitoes collected in different coloured earthen bowls in indoor and outdoor conditions

Experimental place (Oviposit places)	Days	White	Red	Yellow	Blue	Green	Orange	Black	Control
Indoor houses	84	170 ±16.5	161 ±18.93	105 ±13.86	86 ±0.58	165 ±11.15	88 ±18.11	111 ±11.52	136 ±12.63
Outdoor houses	84	65 ±16.26	241 ±16.26	148 ±15.79	116 ±12.03	73 ±16.64	64 ±7.08	189 ±12.77	29 ±7.77
Total number of immature *Aedes* larvae collected within 84 days		235 ±16.38	402 ±17.60	253 ±14.83	202 ±6.60	238 ±13.90	152 ±12.60	300 ±12.15	165 ±10.2

Table 2. Shows that the highest frequency of *Aedes* mosquitoes oviposited (14 times) in White colored bowl followed by Green (12 times) and Red bowls (11 times) as well as the lowest frequency of oviposition was found in Orange bowl (4 times) in indoor. Although in outdoor condition, the highest oviposition frequency of *Aedes* mosquitoes was found in the Red bowl (12 times) followed by the Black (11 times) and Yellow bowls (10 times) as well as lowest oviposition frequency of *Aedes* mosquitoes was found to be 5 times in the Orange bowl.

When compared with indoor and outdoor oviposition frequency in different colored bows, the highest frequency was found in the White colored bowl (14) and followed by the green colored bowl (12) in indoor. In a Red colored bowl of indoor was found slightly lower oviposition frequency than in outdoor and significantly higher in Black bowl of outdoor than in indoor.

When the combination of indoor and outdoor oviposition frequencies, the highest oviposition frequency was found 23 times in Red bowl followed by 20 times each in the White and Yellow bowl and lowest was found in the Orange and control Bowl (9±0.71times). The oviposition frequencies of Green, Black and Blue were seen 19, 18 and 14 times respectively

Table 2: Comparison of the frequency of *Aedes* larvae collected in different coloured earthen bowls in indoor and outdoor conditions

Experimental place (Oviposit places)	Days	White	Red	Yellow	Blue	Green	Orange	Black	Control
Indoor houses	84	14	11	10	6	12	4	7	7
Outdoor houses	84	6	12	10	8	7	5	11	2
Total frequency of immature *Aedes* larvae collected within 84 days		20 ±5.66	23 ±0.71	20 ±0.00	14 ±1.41	19 ±3.54	9 ±0.71	18 ±2.83	9 ±3.54

Table 3 shows that *Ae. aegypti* were found highly oviposit in both indoor and outdoor in all colored earthen bowls. Although *Ae. albopictus* was found to be oviposited only in the black colored bowl in outdoor; it was oviposited five frequencies in the black colored bowl within 84 days. Both *Ae. aegypti* and *Ae. albopictus* gravid females were oviposited together in black colored bowl in outdoor. Fig. 1 shows the highest number of *Ae. aegypti* larvae were collected in the red bowl in outdoor. The species of gravid *Aedes* female laid their eggs in all colored bowls in indoor and outdoor because the larvae were obtained in all colored bowls in both indoor and outdoor. *Aedes albopictus* was collected only from the black colored bowl in outdoor.

Table 3: Indoor, outdoor oviposition and oviposition frequencies of two different *Aedes* mosquitoes in diferent coloured bowls

Colors	Indoor larvae collected	Mosquitoes species				Outdoor larvae collected	Mosquitoes species			
		Ae.aegypti		*Ae. albopictus*			*Ae.aegypti*		*Ae. albopictus*	
		No. of larvae	*No. of frequency*	*No. of larvae*	*No. of frequency*		*No. of larvae*	*No. of frequency*	*No. of larvae*	*No. of frequency*
White	170 ±16.5	170 ±16.5	14	0	0	65 ±16.26	65 ±16.26	6	0	0
Red	161 ±18.93	161 ±18.93	11	0	0	241 ±16.26	241 ±16.26	12	0	0
Yellow	105 ±13.86	105 ±13.86	10	0	0	148 ±15.79	148 ±15.79	10	0	0
Blue	86 ±0.58	86 ±0.58	6	0	0	116 ±12.03	116 ±12.03	8	0	0
Green	165 ±11.15	165 ±11.15	12	0	0	73 ±16.64	73 ±16.64	7	0	0
Orange	88 ±18.11	88 ±18.11	4	0	0	64 ±7.08	64 ±7.08	5	0	0
Black	111 ±11.52	111 ±11.52	7	0	0	189 ±12.77	94 ±12.71	11	95 ±10.88	5
Control	136 ±12.63	136 ±12.63	7	0	0	29 ±7.77	29 ±7.77	2	0	0
Total	1022 ±34.78	1022 ±34.78	71 ±3.02	0	0	925 ±72.41	830 ±66.18	61 ±3.34	95 ±33.59	5 ±1.76

DISCUSSION

Selection of an oviposition site by the female mosquitoes is one of the most important behavioral components of their survival. A female mosquito chooses oviposition sites by a combination of visual and chemical cues. Ovipositing mosquitoes taste the water in a potential oviposition site to detect chemical cues [11]. Further, mosquitoes may also select oviposition sites based on the availability of larval food [24]. Usually, a female does not lay her entire batch of eggs in one location but instead distributes them in multiple water-filled containers, a behavior called skip-oviposition [25]. Gravid *Aedes* females lay their eggs in water-filled containers. However, very few cases have been examined to explain the role of container shape and size in oviposition site selection. *Aedes* mosquitoes are usually day active mosquitoes and might be relying more on optical cues like the contrast between dark container openings for selection of resting and oviposition sites than night active mosquito species [26]. Further, *Aedes* mosquitoes breed in different types of household containers including different colored flower pots. Colton et al.,[25] reported that *Aedes* mosquitoes lay the maximum number of eggs on black ovistrips. Female mosquitoes usually avoid oviposition where interspecific competitors are present [24].

Choice of Ovitrap Color for Oviposition: Seven different colored ovitraps, namely, black, red, yellow, blue, green, orange and white, were chosen in the present experiment for the collection of larvae to determine the *Aedes* mosquitoes under study have the preference for any specific color of the ovitrap for egg laying. The investigation was undertaken both in outdoor (in light) and indoor (in the dark) intending to find out the *Aedes* mosquitoes can differentiate between different colors of ovitraps in outdoor and indoor conditions. It has been observed that *Ae. aegypti* females laid a maximum number of eggs in the white colored ovitrap followed by green ovitrap and lowest number of eggs laid in blue ovitrap in indoor. The numbers of eggs laid on different colored ovitraps in decreasing order were white, green, red, black, yellow, orange and blue in indoor. Although in outdoor *Aedes* mosquitoes laid a maximum of eggs in red ovitrap followed by black ovitrap and lowest number of eggs laid in orange ovitrap. The numbers of eggs laid on different colored ovitraps in decreasing order were red, black, yellow, blue, green, white and orange in outdoor condition. Other researcher observed that *Ae. aegypti* females laid a maximum of 426.67 ± 25.66 eggs in the ovitrap containing black ovistrip in the dark. The numbers of eggs laid in decreasing order of preference were red, yellow, green, and blue, while no eggs were laid in ovitraps containing white ovistrip [27]. Although present study found to be highest larvae in a white color painted bowl in indoor and highest larvae were observed in the red color painted bowl in outdoor.

Same researchers also mentioned that when the *Ae. aegypti* females were allowed to lay eggs in a lighted condition in ovitraps containing the above six different colored ovistrips, a maximum number of eggs (343.33 ± 51.47) were collected in black ovitrap. The decreasing order of preference was red, green, yellow, blue, and white. A minimum of 36.67 ± 10.41 eggs was laid on white ovistrip. Although in the present study found to be female *Ae. aegypti* laid their eggs a maximum number in red ovitrap in outdoor and white ovitrap in indoor. Since *Ae. albopictus* was laid there eggs in high number only in black ovitrap in outdoor. Subrat et al., [27] observed that when the *Ae. albopictus* females were kept in cages containing ovitraps lined with the above six different colored ovistrips, a maximum of 446.67±7.51 eggs were collected from the black ovistrip and a minimum of 35.00 ± 15.39 was recovered from the blue ovistrip in the dark. No eggs were laid by *Ae. albopictus* females in white ovistrip in darkness. Under light condition also the females of this species laid maximum numbers of eggs (328.33±2.89) in black ovistrip. This observation was agreed with the present study, *Ae. albopictus* larvae were observed only in black colored ovitrap in outdoor but not seen in white, red, yellow, green, blue and orange ovitrap in outdoor and also the larvae were absent in all colored ovitraps in indoor in dark condition.

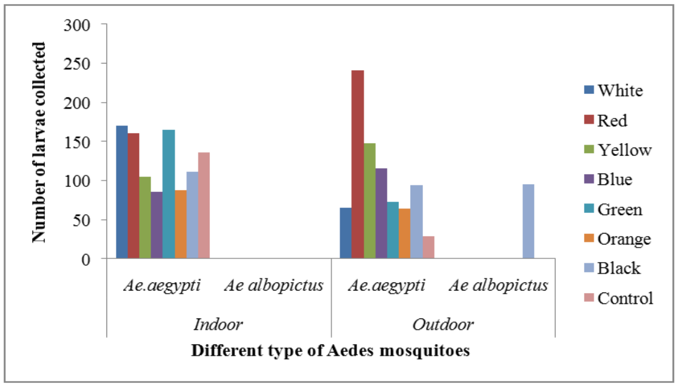

Fig.1. Mean number of *Aedes* larvae collected in different colour of ovitraps in indoor and outdoor condition

Highest frequencies (14 times) of *Aedes* gravid female were oviposited in white colored ovitrap followed by green colored ovitraps and red ovitrap (11 times), and lowest breeding frequency was observed (4 times) in orange within 84 days trial in indoor. In outdoor condition, the highest frequencies (12 times) of *Aedes* gravid female were oviposited in red ovitrap followed by black ovitrap (11 times), and lowest breeding frequency was observed (5 times) in orange ovitrap. Although when combined indoor and outdoor the highest breeding frequency (23 times) was found in red ovitrap followed by the white and yellow trap (20 times each) and lowest frequency was observed (9 times) in orange and control ovitrap. The same result has been found by Kumawat et al., [28] in India, they revealed that red colored ovitraps have highest positivity (92.7%), followed by black and orange (91.7% each), green (76.3%) and transparent (45.8%).

Epidemiologically, gravid females are the most important component of the mosquito population and are targeted in mosquito control programmes in active surveillance of disease for the early detection of epidemic events. Field mosquitoes are also collected using the ovitrap technique, the cheapest and easiest method for collecting *Aedes* mosquitoes [29]. Fay and Perry [30] were first to use ovitraps for *Ae. aegypti* (Linn.) surveillance and Fay and Eliason demonstrated that the ovitrap was in some aspects superior to larval surveys [31]. Ovitraps were also shown to be useful sampling devices in determining *Ae. aegypti* distribution and seasonal population fluctuation [32]. Ovitraps are relatively easy to construct and are sensitive in detecting the presence of gravid females, even at low population densities, making them ideal for surveillance and control of vector species [33]. The studies have demonstrated the advantages in time and labor savings and increased sensitivity in using artificial oviposition devices as compared to various adult traps for surveillance of *Ae. aegypti* [32,33]. The addition of insecticides or insect growth regulators to the traps renders them lethal ovitraps [34] Behavioural choices of female mosquitoes are clearly affected by color, either as a substrate or as light. Snow mentioned that *Ae. aegypti* were most sensitive to green-orange light (470–610 nm) and thus avoided those colors while seeking more cryptic oviposition sites [35]. Present study observed that high number of *Ae. aegypti* larvae were breed in the green colored bowl followed by the red bowl in indoor. Earlier studies have also exhibited that black containers are the most attractive colored targets for male [30] and female [36] mosquitoes. Similarly, in present studies in outdoor also found that black ovitraps are more attractive to *Ae. albopictus* mosquitoes. Another study in Malaysia, gravid *Ae. albopictus* in rural habitats oviposited more in red and black ovitraps than in blue, yellow, green, white, and plain (unpainted) ovitraps [37]. In the present study, gravid female *Ae. albopictus* laid only in the black colored bowl in outdoor. A laboratory studies, gravid females of *Ae. albopictus* laid significantly more eggs in black, red, and blue ovitraps than in green, yellow, white, or clear (unpainted glass) ovitraps [37], which correlated well with findings of Snow's [35] for color preferences of gravid *Ae. aegypti*. Another researcher also reported the preference for dark colored glass jars, especially black, blue, and red ones over light colored jars by *Ae. albopictus* mosquitoes during their study [37]. Hoel et al., [38] tested five choices of colors against *Ae. albopictus* in Florida and based on the mean eggs collected, the choices of colors were black > blue > checkered > orange > striped and white. Like our studies, they also found the preference of black color more than the other competing colors and also observed the positive response of gravid *Ae. albopictus* to black and orange targets and concluded that orange is perceived as an attractive stimulus. Different researchers demonstrated that orange lighted targets attract host seeking *Ae. albopictus* and also reported that *Ae. aegypti* can detect orange light based on the electroretinographic examination and also revealed spectral sensitivity ranging from UV to orange colored light. [39].

CONCLUSIONS

In conclusion, the female mosquito of *Ae. aegypti* species laid the maximum number of eggs in the white colored bowl and minimum in the blue colored bowl in indoor and maximum in red colored bowl and minimum in the orange colored bowl in outdoor. *Aedes albopictus* species laid only in the black colored bowl in maximum number in outdoor. *Aedes albopictus* larvae were lacked in all colored bowl and control bowl in indoor and outdoor except black colored pot in outdoor. The black color is most attractant color for *Ae. albopictus* gravid female and red and white color are the most attractant color for *Ae. aegypti* in outdoor and indoor. Although both *Ae. aegypti* and *Ae. albopictus* gravid females were oviposited together only in black colored bowl in outdoor. They usually preferred to lay eggs in ovitraps containing distilled water only. Further, when combined indoor and outdoor, *Aedes* females put more number of eggs in decreasing order showed red>black>yellow>green> >white>blue>orange and control ovitraps. Usually *Ae. albopictus* mosquitoes avoided all colored of containers for oviposition but preferred black color ovistrip for laying eggs. All colored bowls were favorable for the oviposition for *Ae. aegypti*. Further study of control tools and strategies could be possible to design and develop suitable colored ovitrap for the control of *Aedes* mosquito vectors.

ACKNOWLEDGMENT: None

CONFLICTS OF INTEREST

The authors have reported no conflict of interest.

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