Bacillus thuringiensis var. *israelensis* Misting for Control of *Aedes* in Cryptic Ground Containers in North Queensland, Australia

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Abstract. In Australia, dengue is not endemic, although the vector mosquito *Aedes aegypti* is established in far north Queensland (FNQ). *Aedes albopictus* has recently invaded the Torres Strait region, but is not established on mainland Australia. To maintain dengue-free, public health departments in FNQ closely monitor introduced dengue infections and confine outbreaks through rigorous vector control responses. To safeguard mainland Australia from *Ae. albopictus* establishment, pre-emptive strategies are required to reduce its breeding in difficult to access habitats. We compare the residual efficacy of VectoBac WDG, *Bacillus thuringiensis* var. *israelensis* (Bti) formulation, as a residual treatment when misted across a typical FNQ bushland using a backpack mister (Stihl SR 420 Mist Blower) at two dose rates up to 16 m. Semi-field condition results, over 16 weeks, indicate that Bti provided high mortality rates (> 80%) sustained for 11 weeks. Mist application penetrated 16 m of dense bushland without efficacy decline over distance.

INTRODUCTION

Dengue is recognized as the world's most important emerging tropical arboviral disease.¹ Its global resurgence makes it a major public health problem in the tropics and sub-tropics.² Dengue reappeared in far north Queensland (FNQ), Australia during 1981, after an absence of over 26 years.³ Since the 1981 outbreak, it has been reintroduced annually as small incursions initiated by viremic travelers; each incursion leading to an outbreak of dengue disease of varying intensity.^{4,5} To remain dengue-free, mosquito management responds to outbreaks by conducting targeted *Aedes aegypti* (Linn.) control in areas with potential and active dengue transmission. Tight vector control is essential to ensure FNQ remains free of endemic dengue disease, despite the permanent presence of vector mosquitoes and repeated incursions from viremic travelers.⁶

In Australia, the principal dengue vector mosquito *Ae. aegypti* exists only in the northern Queensland FNQ^{3,7}; however, a secondary dengue vector *Aedes albopictus* (Skuse), the Asian tiger mosquito, is now considered endemic in the Torres Strait,⁸ the series of islands between FNQ and Papua New Guinea. *Aedes aegypti*, is anthropophilic, feeding almost exclusively on humans,⁹ and as such has a close association with urban settings, preferentially using water-filled artificial containers as larval habitat such as tires, buckets, birdbaths, and pot plant bases.^{10–12} During dengue disease outbreaks, mosquito management in FNQ controls *Ae. aegypti* only in domestic urban environments. *Aedes albopictus* does not feed exclusively on humans and its larvae are found further from human settlements in natural reservoirs such as palm fronds, coconut shells, and leaf litter, as well as artificial containers.¹³ Because insecticide treatments require locating and treating potentially thousands of containers, many control programs for *Ae. albopictus* focus on source reduction rather than insecticidal use.^{14,15} Should *Ae. albopictus* populations move south, introducing a competent dengue vector that breeds and feeds in sylvan habitat, independent of humans, the balance could easily be tipped to “unmanageable.”¹⁶

One potential method to control mosquito production in hidden receptacles is through mist application of a residual larvicide. *Bacillus thuringiensis* var. *israelensis* (Bti) is an established microbial agent that kills mosquito larvae when ingested.^{17,18} It is target-specific, free from bacterial contaminants and exotoxins, safe for drinking water, and poses no significant hazards to the environment.^{14,19} Furthermore, it is effective against dengue vectors in village, urban, and suburban environs, and it has recently been reported as reducing dengue cases and vector numbers.¹⁹ To date, *Aedes* resistance to Bti has not been reported,¹⁸ and lethal application of Bti in treated containers does not repel *Aedes* from ovipositioning.¹⁴

VectoBac WDG (active ingredient = *Bacillus thuringiensis* var. *israelensis*, strain AM 65-52, 3,000 International Toxic Unit ITU/mg) presently is the only formulation evaluated with specifications published by the World Health Organization (WHO).²⁰ Generally, Bti is used for broad acreage treatments; it poses virtually no threat to non-target organisms.¹⁹ Some studies have shown residual efficacy controlling *Ae. aegypti* in water-storage containers for up to 16 weeks.^{21,22} During field trials in Cairns, megadoses of VectoBac WDG demonstrated over 90% residual control of *Ae. aegypti* in container habitats, up to 24 weeks.²³ Distance applications have also been tested using ultra low volume (ULV) Bti spraying. This method penetrated tires in vegetation up to 30 m away resulting up to 45% mortality of *Ae. aegypti* larvae after 7 days.²³ One study examining *Ae. albopictus* control in a Singapore forest using second weekly Bti misting reported larval densities decreased from 27.9 to 3.2 per ovitrap over 3 months.¹³ To date, no study has tested the direct single application of Bti on potential *Ae. albopictus* larval habitats, comparing efficacy over distance and time. To reduce *Ae. albopictus* breeding potential in hidden containers, we examine the effective range and duration of Bti residual larvicidal control when applied as a single application by a backpack mister for mosquito control, in a bushland setting.

MATERIALS AND METHODS

Site description. The Torres Strait Islands (9.9°S 142.6°E), situated almost directly north of Cairns (16.9°S 145.8°E), offer a similar climate to Cairns with a mean maximum temperature of 29.4°C compared with Cairns 29.0°C, and an

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FIGURE 1. Misting at study site near Cairns Australia, across measured distances.

average rainfall of 1,772 mL/pa compared with Cairns 2,022 mL/pa based on long-term averages for Horn Island.²⁴

We conducted a pilot study and a misting trial in a Cairns bushland setting representative of a typical Torres Strait Islands sylvan environment, featuring medium density trees with sparse undergrowth (Figure 1). At the site canopy height averaged 10–15 m with scattered emergent trees (*Acacia* spp., *Terminalia* spp., and *Melaleuca* spp.) to 15–20 m high. Vines occupied gaps in the canopy and ground cover was relatively sparse. The study site included an area of ~150 × 23 m, bordered by a dirt track (Figure 1).

Pilot trial. To determine experimental distances, a pilot spray trial was conducted before the main misting trial. A site downwind of the main misting study was selected and three 20 m transects were marked out perpendicular to the track. Test cups (clear plastic, 250 mL disposable drinking cups) and moisture-sensitive dye cards (Spraying Systems Co. Pty. Ltd., Australia) were placed at 1 m intervals along each transect. A backpack mister, Stihl SR 420 Mist Blower (Stihl Pty. Ltd., Australia) set at dial 2 delivered flow rates as determined by molecular weight and viscosity of solution, thus the actual flow rate is confirmed retrospectively from remaining volume. VectoBac WDG was mixed and applied to the trial plot at the dose rate of 400 g/ha (minimum label recommendation).²⁵ Misting application, spraying horizontally perpendicular to transect was timed to ensure consistent application, (Figure 1).

Treatment and weather details during misting are shown in Table 1. The pilot study was conducted on 20 April 2008.

Droplet profile. To determine the droplet profile, magnesium oxide (MgO)-coated slides were placed at 2 m intervals from the spray line, for 16 m (Table 2). This portion of the study was conducted at the Institute for Medical Research, Malaysia. The remaining volume in backpack mister unit, Stihl SR 420, confirmed a discharge of 500 mL/min (dial 2).

Misting trial. Pilot trial dye card records (Figure 2) was used to determine optimal experimental distances. Bioassay cups were placed along transects for Bti misting. Distances 2, 4, 8, 12, and 16 m from the track were selected as the treatment distances along the three transects located 5 m apart. Vegetation density was assessed between treatment areas. A white cloth (2 × 1 m) held vertically was used to estimate the foliage density (as percent of sheet occluded by vegetation) at each of the five distances along transect.

Two treatment dosages—400 g/ha and 800 g/ha, representing the minimum and maximum recommended label rates, were applied to two sites separated by 45.5 m of bushland, commencing on 21 April 2008. Within each treatment area three plots were marked out, each containing three 16 m transects. Test cups (250 mL) were labeled and placed on the ground along each transect at distances of 2, 4, 8, 12, and 16 m from the track. Thus, 45 cups were positioned for each dose, one cup at each of the five distances, along three transects, replicated in three plots (5 × 3 × 3 = 45).²³ Dye cards were placed at each of the five distances within each plot, but only along one transect, for a total of 15 dye cards per treatment dose.

The same backpack mister was used as with the pilot trial, with the flow rate recalibrated to 470 mL/min with the spray intensity dial set at 2. VectoBac WDG was mixed and applied to the two treatment areas at the dose rates of 400 g/ha and 800g/ha. The backpack mister was rinsed thoroughly between treatments. Details of treatment dose and application are outlined in Table 1. The areas were treated at 7:35 AM and 9:20 AM, 21 April 2008. After misting, the test cups were left undisturbed for 30 minutes before being fitted with a lid and collected. They were placed in respective treatment crates covered with mosquito-proof mesh similar to that used by Ritchie and others (2010) and transported¹⁷ to a shaded outdoor area that was exposed to rainfall. This placement enabled controlled conditions for rearing, thus, reducing the influence of random environmental effects to the study. On the day of the misting (Week 0), the cups were filled with 200 mL of water and a single longan (*Dimocarpus longan*) leaflet was added as a food source. Ten 2nd-3rd instar *Ae. aegypti* larvae were

TABLE 1
Treatment and weather* details for the main misting trials

	Pilot trial	Main trial	
Dose rate	400 g/ha	400 g/ha	800 g/ha
Mister flow rate (dial 2)	400 mL/min†	470 mL/min†	470 mL/min†
Treatment area size	35 × 20 m (700 m ²)	40 × 20 m (800 m ²)	40 × 20 m (800 m ²)
Mix ratio for backpack mister	200 g/10 L	170.2 g/10 L	340.4 g/10 L
Walking pace	10 m/min	10 m/min	10 m/min
Duration of misting	3.5 min	4 min	4 min
Mean temperature (°C)	25.1	23.7	23.7
Maximum temperature (°C)	26.2	29.4	29.4
Minimum temperature (°C)	20.9	20.4	20.4
Mean relative humidity (%)	68.9	71.4	71.4
Mean wind speed (m/s)	1.38	0.73	0.73

* Rainfall totaling 113.8 mm fell during the study period at meteorological station 10 km SE of the site.

† Mister set on dial 2, delivered a varying flow rate that was confirmed after the experiment by remaining volume.

TABLE 2

Droplet profile on magnesium oxide slides, volume median diameter (VMD), for 400 g/ha and 800 g/ha VectoBac WDG Spray, mean ($\pm$ SE)

Distance (m)	VMD μ m 400 g/ha	VMD μ m 800 g/ha
2	95.37 $\pm$ 11.11	165.17 $\pm$ 24.24
4	38.94 $\pm$ 5.65	114.87 $\pm$ 16.55
8	62.21 $\pm$ 3.83	86.17 $\pm$ 29.26
12	53.93 $\pm$ 9.11	60.39 $\pm$ 19.68
16	NA*	47.97 $\pm$ 13.11

*Droplets were not observed for 400 g/ha spray at 16 m from spray line.

placed in each cup. After 24 hr and again at 48 hr the number of larvae alive was recorded and all live larvae were removed. The test cups were challenged weekly (21 April–11 June 2008) with 10 2nd-3rd instar *Ae. aegypti* per cup, and single longan leaflet added to each cup fortnightly to simulate leaf fall into containers.

Statistical analysis. We compared Bti in two strengths (400g/ha, 800g/ha) when misted to five distances (2, 4, 8, 12, 16 m) across three plots, with three transects for each plot over 16 weeks (0–15). Data were counts (dead mosquito larvae) measured at 48 hr (for Weeks 0–2 only 24 hr data were available) and a total of 10 dead larvae were placed in each cup. The results, (counts/10) are a proportion and were modeled assuming a binomial distribution. Odds ratios (ORs) are presented instead of coefficients for ease of comparison. The OR indicates the strength of association of the dependent variable to each independent variable, with *P* values indicating statistical significance. Data were modeled together to initially identify significant associations, after which two models were created separately by dose (high/low) to best identify the point at which larval mortality declined, and duration of effect (week). All analyses were performed using STATA version 11.0 (Stata Corp., College Station, TX).

RESULTS

Pilot trial. The pilot trial results (Figure 2) indicate that misting traveled at least 20 m in a vegetated site, however,

droplet counts were much lower beyond 16 m and 48 hr larval mortality rates were inconsistent beyond 16 m (Figures 2 and 3). The droplet distribution from the pilot trial was used to establish experimental distances. Distances 2, 4, 8, 12, and 16 m from the track were subsequently selected as the treatment distances along the three transects located 5 m apart.

Droplet profile. The mean droplet size for 800 g/ha spray was compared with 400 g/ha spray (Table 2). Droplet analysis revealed that the higher viscosity spray mix (800 g/ha) with double the amount of Bti applied using the same discharge rate as 400 g/ha resulted in varied droplet size between the dose rates. The droplet profile indicates that 400 g/ha droplets were smaller than 800 g/ha across all distances, and droplet size decreased within each dose as distance increased. Droplets were not observed for 400 g/ha spray at 16 m from the spray line.

Misting trial. The density of vegetation at each transect distance visible through a white sheet was not significantly different across treatment areas (*t* tests, *P* values ranged from 0.35 to 0.97, data not shown). Plant species and layout were also consistently similar between the low and high dose treatment areas (based on visual comparison, statistical analysis not performed).

The cumulative mortality over the first 8 weeks of treatment indicated that misting killed 80.5% (95% CI: 78.9–82.1) of 2nd-3rd instar *Ae. aegypti* to a distance of 16 m. The mortality rate at 8 weeks post treatment was 74.7% (95% CI: 67.9–81.3) at low dose, and 79.8% (95% CI: 73.2–86.3) for high dose application.

The binomial model results indicate that there was no difference in larvae mortality between 800 g/ha and 400 g/ha misting (*P* = 0.66, Table 3; Figure 4). Larval mortality was not statistically significantly associated with distance (*P* = 0.96), indicating that mortality was equally high at 16 m compared with 2 m. Plot and transect were not independently associated with larval mortality, indicating a robust study design plot (*P* = 0.78) and transect (*P* = 0.63). Standard diagnostics revealed a robust model, with normally distributed residual errors versus fitted values. Larval mortality

FIGURE 2. Mean ($\pm$ SE) number of droplets (all sizes) per cm² on moisture-sensitive dye cards set at 1 m intervals along three transects for the pilot trial.

FIGURE 3. Mean ($\pm$ SE) *Aedes aegypti* larval mortality rates after 24 hr in test cups set at 1 m intervals along three transects for the pilot trial.

significantly declined over time (weeks) ($OR = 0.93$, $P < 0.001$), indicating a loss of effect over time, which may be dose dependent (Table 3; Figure 4). The drop in mortality Week 2 (Figure 4, Table 4, $OR = 0.62$, $P = 0.06$) relative to the reference week, is most likely indicative of delivery error—overshooting during application, this was not statistically significant. It is unlikely that rainfall influenced this drop, as total rainfall measured during this period was < 5 mm/week from Weeks 0–4, compared with 27.8 mm during Week 5, with no apparent drop in mortality.

To identify the duration of effect of misting offered between high and low dose Bti, two further models were created that compared weekly larval mortality with the reference week (Week 0). These results similarly indicated that distance (2, 4, 8, 12, 16 m) was not associated with a statistically significant reduction on larval mortality, nor were plot or transect. Time (week) was associated with a significant reduction in larvae mortality after Week 9, which was consistently lower after Week 11 for 400 g/ha, and consistently lower after Week 11 for 800 g/ha Bti (Tables 4 and 5). The tight CIs around the mean mortality rates (Figure 4) indicate that mortality per cup was not highly varied.

DISCUSSION

This study shows that Bti application by a backpack mister can be used to control *Aedes* breeding in small, hidden container habitats in a vegetated, non-domestic bushland setting.

TABLE 3

Binomial model of dead larvae/10 comparison between low and high dose Bti treatments, over distance and time*

	OR	SE	P	95% CI
800g/ha vs. 400g/ha (ref)	1.03	0.07	0.66	0.90–1.18
Distance	1.00	0.01	0.96	0.99–1.01
Week	0.93	0.01	< 0.001	0.92–0.95
Plot	1.01	0.04	0.78	0.93–1.10
Transect	0.98	0.04	0.63	0.90–1.06

*OR = odds ratio; CI = confidence interval.

Doses of 400 g/ha and 800 g/ha provided high larval ($> 80\%$) mortality, and this effect was sustained for up to 9 weeks post-misting. Furthermore, our mist application was able to penetrate 16 m of dense bushland, with no statistically significant difference between the distances (2, 4, 8, 12, 16 m).

Our results are comparable to those of Lam and others¹³ who applied 500 g/ha Bti every second week for 12 weeks (6 cycles), to a bushland setting in Singapore. Lam and others found that backpack administration of Bti (VectoBac WDG) significantly reduced adult *Ae. albopictus*, efficacy reductions were reported as 53.2% and 80.0% at the Bti treatment site during the second and third month, respectively, when compared with the untreated bushland setting (control).¹³ Lam and others examined the larvicidal efficacy of Bti by two measures, larval density, and *Ae. albopictus* adult activity using an ovitrap index. Both measures indicated a reduction in *Ae. albopictus* in a natural setting.¹³ The methodology of Lam and others differed from our recent study as Bti was applied every second week, not as a single application, thus the residual effect of a single application was not investigated; furthermore, the distance of application was not analyzed for larvicidal efficacy. Additionally, Lam and others¹³ suggest that wide swath spray applications are required in these situations.

One study from Malaysia targeting *Aedes* breeding sites reported that a spray distance of 30 m could be obtained when using a vehicle mounted ULV sprayer with a flow rate of 300–500 mL/min.²³ This is a substantial distance, even when obtained in an open area rather than dense bushland. Results from our pilot study indicate that Bti could be applied using the mister for distances greater than 16 m in a bushland setting, but efficacy was diminished. Although our application distance was shorter, our Bti application technique allowed for longer Bti residual protection up to 11 weeks. This compares with Lee and others²³ findings that were sustained for a period of 14 days. A droplet comparison between the two studies indicates that ULV spraying achieves small droplets (20–50 μ m), whereas our misting droplets were comparatively larger at mean 38–170 μ m.²³ The smaller size of ULV application facilitates

FIGURE 4. Mean larval deaths at 48 hr between low versus high dose Bti treatments over time, with 95% confidence interval (CI).

propulsion and dispersal of droplets. Our relatively larger size droplets inhibit travel, 16 m compared with up to 30 m as reported using ULV Bti application.²³ This is also caused by delivery by Stihl SR420 operating at 3.5 hp, whereas IGEBA U15 reports 11 hp engine output. However, the corollary of a larger droplet size is greater residual protection, as larger droplets contain a higher Bti dose.²³ Our results also indicated that 800 g/ha applications provided larger droplets than 400 g/ha, when sprayed from the same backpack mister. Thus, the trade-off for shorter application distance was longer residual control.

TABLE 4

Binomial model of dead larvae/10 for 400 g/ha BTi treatment, over time*

	OR	SE	P	95% CI
Distance	1.00	0.01	0.71	0.99–1.02
Plot	1.06	0.06	0.35	0.94–1.19
Transect	0.98	0.06	0.74	0.87–1.10
Week 0	Reference			
Week 1	0.88	0.20	0.59	0.56–1.39
Week 2	0.62	0.15	0.06	0.38–1.01
Week 3	0.86	0.20	0.50	0.54–1.35
Week 4	0.85	0.20	0.47	0.54–1.33
Week 5	0.74	0.18	0.20	0.46–1.18
Week 6	0.88	0.20	0.57	0.56–1.38
Week 7	0.70	0.17	0.14	0.43–1.12
Week 8	0.74	0.18	0.21	0.46–1.19
Week 9	0.48	0.13	0.01	0.29–0.82
Week 10	0.70	0.17	0.14	0.43–1.12
Week 11	0.42	0.12	< 0.001	0.24–0.73
Week 12	0.47	0.13	0.01	0.28–0.80
Week 13	0.39	0.11	< 0.001	0.22–0.68
Week 14	0.88	0.20	0.59	0.56–1.39
Week 15	0.62	0.15	0.06	0.38–1.01

*OR = odds ratio; CI = confidence interval.

Tan and others¹⁹ combined the methods of Lam¹³ and Lee²³ and applied them to a residential area in Selangor, Malaysia.¹⁹ Over a 38-week period, Bti was misted throughout a residential area using the Stihl SR 420 backpack mister set on various gauge settings, every 2 weeks findings reported indicate that ovitrap index was suppressed to 10% during the four weeks post-exposure, and this compared with the control site reporting ovitrap index over 40% during the study period.¹⁹ Furthermore, actual dengue infections were lower in the treated area, one case reported from the treatment area, compared with 15 from the control site (across the road) during the same period.

TABLE 5

Binomial model of dead larvae/10 for 800 g/ha BTi treatment, over time*

	OR	SE	P	95% CI
Distance	1.00	0.01	0.66	0.98–1.01
Plot	0.97	0.06	0.59	0.86–1.09
Transect	0.98	0.06	0.72	0.87–1.10
Week 0	Reference			
Week 1	0.85	0.20	0.47	0.54–1.33
Week 2	0.74	0.18	0.21	0.46–1.19
Week 3	0.87	0.20	0.53	0.55–1.36
Week 4	0.68	0.17	0.12	0.42–1.10
Week 5	0.77	0.18	0.28	0.49–1.23
Week 6	0.80	0.19	0.35	0.51–1.27
Week 7	0.75	0.18	0.23	0.47–1.20
Week 8	0.80	0.19	0.34	0.50–1.27
Week 9	0.60	0.15	0.04	0.36–0.98
Week 10	0.65	0.16	0.08	0.40–1.06
Week 11	0.59	0.15	0.04	0.36–0.97
Week 12	0.57	0.15	0.03	0.35–0.95
Week 13	0.45	0.12	< 0.001	0.26–0.76
Week 14	0.13	0.06	< 0.001	0.06–0.30
Week 15	0.19	0.07	< 0.001	0.09–0.40

*OR = odds ratio; CI = confidence interval.

Differences between their methodology and ours include the application to the residential area with repeated application,¹⁹ and distance of application was not recorded. Similarities include the application of Bti by a Stihl mister for vector control in cryptic containers.

One limitation to this study includes the transfer of cups from the field site to a crate covered with screen and shade cloth set outdoors. This technique was adopted to reflect field conditions while monitoring in a controlled environment.²³ The shade cloth allowed exposure of containers to rainfall, and ambient temperature fluctuations, however UV exposure is reduced, which has been reported as reducing the efficacy of Bti.^{17,23,26} When used in standard deployment conditions VectoBac WDG may be subjected to higher temperatures, more sunlight, and potentially more flooding situations, which may reduce the larvicidal qualities of VectoBac WDG.¹⁷ Further field experimentation to determine impacts of these environmental factors in our situation is logistically unviable.

For *Aedes* control, in particular *Ae. albopictus* in a bushland setting, we require the application of a liquid to facilitate dispersion to distances up to 16 m, and one that offers residual control after a single application. Granular preparations may offer long-term duration of residual control, lasting up to 360 days for methoprene pellets in bromeliads while sustaining 100% efficacy; however, these cannot be applied through dense bushland over a distance.^{15,17} And, dry application of larvicides by hand into individual containers is an unsuitable approach for *Ae. albopictus* that use small, cryptic receptacles such as leaf litter and coconut shells in a bushland setting.¹³ The ULV dispersal can target "micro-habitats" in vegetated areas, they can also offer greater distance,¹⁹ although smaller droplet size reduces the dose effect, thus residual efficacy is lower. Here, we have tested a single application of Bti (VectoBac WDG) for mosquito control applied using a backpack mister, and this combined treatment/application technique, fills the gap for pre-emptive *Ae. albopictus* control in a bushland setting.

CONCLUSION

We have shown that Bti, VectoBac WDG can be applied successfully using a mist application in a dense bushland setting up to 16 m. Discrete containers that would otherwise be logistically impossible to locate for source reduction, can be treated using Bti and have a residual effect for up to 9 weeks in FNQ field conditions. Findings in this study indicate there was no significant difference between the two Bti concentration rates, the use of 400 g/L would provide sufficient dose to control *Ae. aegypti* larvae, up to 9 weeks. However, 800 g/ha provided slightly greater residual control, as indicated by higher larval mortality rates through to Week 11, over 2 months post-misting. With this in mind, mosquito control operators could choose a dose rate determined by budget and operational logistics, with the higher dose appropriate for situations where control operators have infrequent access to the site. Decisions on treatment swath width would need to be assessed for each site depending on vegetation density and type and site access. This application could be used for other container breeding *Aedes* mosquitoes including *Ae. albopictus*.

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