
Development and Testing of Insecticide Bait Formulations for Enhanced Bioefficacy Against Queensland Fruit Fly

Amin Khan Pathan^{1, *}, Dave Geoffrey Voice², Richard Arthur Ian Drew³, Dave John Saville⁴

¹Ministry for Primary Industries, Rotorua, New Zealand

²Ministry for Primary Industries, Christchurch, New Zealand

³International Centre for the Management of Pest Fruit Flies, Griffith University, Brisbane, Australia

⁴Saville Statistical Consulting Limited, Lincoln, New Zealand

Email address:

amin.pathan@mpi.govt.nz (Amin Khan Pathan)

*Corresponding author

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Abstract: Protein baiting has always been an essential component of fruit fly eradication programmes following detection of a breeding population in New Zealand. In 2015, a breeding population of Queensland fruit fly (*Bactrocera tryoni*) was successfully eradicated in Grey Lynn, Auckland after implementing a baiting programme. Although the baiting programme appeared to be successful at the time, improvements were sought in terms of better adhesion and retention of bait spots on foliage, reduction of bait application volume of individual spots, reduction in off target drift and safety improvements without compromising bioefficacy. At the time, concerns were raised by regulatory authorities and members of public in urban areas on the reliance on a single insecticide, as well as on the low viscosity of the bait mix. These concerns needed to be addressed to maintain regulatory and social licenses to undertake bait application in future responses. In order to improve the viscosity of the bait solution, a xanthan gum-based additive (keltrol) was added to the bait formulation (natflav) mix and tested with both the incumbent insecticide (fipronil) and a relatively new insecticide (spinetoram) to optimise efficacy and longevity of the insecticides against Queensland fruit fly *Bactrocera tryoni* (Froggatt) (DIPTERA: Tephritidae). Results showed that keltrol significantly enhanced the bioefficacy through significant increases in the uptake of insecticide via oral route and that spinetoram at twice the label rate could be considered as a useful and safer alternative to Fipronil in protein bait mixes.

Keywords: Queensland Fruit Fly, Protein Bait Application, Bioefficacy, Eradication Response, Fipronil, Spinetoram, Keltrol Gel, Xanthan Gum

1. Introduction

Fruit flies from the family Tephritidae are high impact pests of horticulture worldwide that attack a range of fruit and vegetable species of commercial importance. Ongoing fruit fly out-breaks in Australia, and border and post border detections in New Zealand since 2012 indicate that fruit flies continue to pose a significant threat to New Zealand's horticultural industries.

Queensland fruit fly (QFF) is considered the most damaging pest to Australia's horticulture industries [1-3] and outbreaks of this species in Australian commercial fruit production farms

have been ongoing for more than a century [4]. Over the past two decades in Australia, QFF has expanded through southern New South Wales and northern Victoria.

There were further outbreaks in Flinders Island north east of Tasmania and northern Central Tasmania in 2017. An increase in QFF populations and its spread into previously pest-free areas of Australia were recognised as an increase in potential threat of QFF host material arriving in New Zealand that could potentially carry eggs or larvae through the common import pathways [5]. Passenger-carried undeclared fruit was identified as the main pathway of concern by MPI that would likely be the cause of a successful incursion of

this damaging pest in New Zealand.

In the worst-case scenario of finding a fruit fly breeding population in the heart of kiwifruit production area Te Puke in the Bay of Plenty, an impact to the magnitude of \$430 million dollars in the form of quarantine export costs alone was estimated [6]. To manage the risks presented by future detections and incursions of fruit fly in New Zealand, the New Zealand Ministry for Primary Industries (MPI) needs to ensure it continually builds on information and lessons learnt from previous fruit fly responses and overseas fruit fly management practices. It is essential that incursant populations are quickly brought under control, not only to prevent the spread, but also to keep export and other restrictions (including eradication costs) to a minimum.

Once the young flies emerge from their puparia and crawl through the topsoil, the flies seek out and feed on protein on leaf and fruit surfaces, especially on host fruit plants. Protein promotes growth to sexual maturity and after the female flies develop eggs, they lose some interest in this food source and begin to lay eggs. Protein is highly effective when sprayed on to foliage to attract fruit flies, particularly immature females. Trapping, baiting, fruit monitoring and fruit collection are essential elements of fruit fly eradication programmes in New Zealand MPI Fruit Fly Response standards and procedures.

In February 2015, MPI responded to a QFF incursion (*Bactrocera tryoni*) in the Auckland suburb of Grey Lynn. As part of the management operations, insecticide bait was applied to fruiting trees and plants within a 1.5 km radius or from the original detection site which included nearly 10,000 properties. This bait contained a specific protein to attract both male and female protein hungry fruit flies. Addition of a low concentration of fipronil insecticide in the bait mixes ensures delivery of a lethal dose via oral pathway to fruit flies. In line with the then MPI Fruit Fly Standard specifications, the baits spots were applied using knapsack sprayers and dispensed as 100 ml large spots using a coarse nozzle. While all measures were undertaken to minimise inadvertent bait deposition and the resultant drift issues, the watery nature of the bait mix, the relatively large volume of individual bait spots (ca. 100 ml), and the observed inefficiencies in the delivery method (knapsack sprayer fitted with a coarse nozzle) identified the need for improving the bait formulation and/or application systems to provide better deposition on target foliage while minimising off-target contamination.

Prior to 2015, the last fruit fly response that required organism management operations involving insecticide application in New Zealand was in May 1996 [7]. Between 1996 and 2015, there has been significant changes in terms of social acceptance of insecticide (insecticide class and delivery methods) in urban environments, health and safety standards, conditional regulatory approval requirements, as well as development of new more efficient formulation and application systems. MPI needed to ensure that the most up to date insecticide formulation and application technologies and solutions were available for use in fruit fly eradication programmes with maximum efficiency and safety, while meeting all regulatory concerns and requirements to maintain

safety standards and social license to operate. The QFF 2015 response created opportunities for enhanced preparedness, innovation and capability building within MPI that can be pursued further for use in future organism management programmes. As a consequence, a 'Bait Improvement Project' was initiated in March 2016 with the aim to identify insecticides that could complement or supplement insecticide used in previous fruit fly responses, and to enhance the efficacy (application efficacy as well as bioefficacy) of selected insecticides to achieve optimum safety and lethality from bait applications.

The current study was undertaken with the following objectives:

- 1) to determine the mortality at 24 and 72 hours of Queensland fruit fly exposed to selected protein: bait insecticide combinations;
- 2) to determine the persistence (efficacy over time) of selected protein bait insecticide combinations over a 6-day period of weathering on leaves;
- 3) to identify a safer and socially acceptable alternative to existing insecticides used in fruit fly control/eradication without compromising bioefficacy.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Material

Experiments were conducted on orange (*Citrus sinensis* Linnaeus) cv Valencia trees, 2m high and 3-4m in diameter. Branches were labelled for each treatment and 6-8 leaves at the end of each branch were dipped in each treatment solution to ensure uniform coverage of the solution (Figure 1).



Figure 1. Orange trees and leaf dip treatments.

2.1.2. Chemicals

Fipronil was supplied as Maestro 200SC (Nufarm Australia Limited), spinetoram as Success™ Neo 120SC (Dow Agrosciences, Australia), protein bait as Natflav® 500

(Denco Trading Pty Ltd, Australia), and xanthan gum-based gel as keltrol (Bugs for Bugs Private Limited, Australia; <https://bugsforbugs.com.au/product/fruit-fly-lure-thickener/>).

Keltrol is a food grade and technical grade additive used mainly as food additive and drilling additive in food, personal care, pharmaceutical, industrial and animal feed applications. Its main ingredient is a xanthan gum, which is a natural polysaccharide and Xanthan gum is a hydrocolloid, a substance that disperses in water and provides a thickening or gelling effect by increasing the viscosity of a solution. Even at low concentrations xanthan gum solutions show a high degree of viscosity in comparison with other polysaccharide solutions. This property makes it a very effective thickener and stabiliser. Keltrol is commercially available with several synonyms (https://www.chemicalbook.com/ChemicalProductProperty_EN_CB3735028.htm).

Natflav was developed more than 20 years ago and is considered as the premium protein bait for use in fruit fly management programs. The yeast bait product contains 420 g/L protein material and designed to attract and kill fruit flies when used as an attractant in a baiting mixture. The product

is now acquired and marketed as Fruition® Natflav® 500 by Food Industry Products Pty Ltd. Natflav; <https://agnova.com.au/products/natflav.html>).

2.1.3. Adult Fruit Flies

Adult *Bactrocera tryoni* were produced from a laboratory colony maintained for 20 generations at Griffith University (Nathan Campus, Brisbane, Australia). Test flies were all deprived of protein and used for the experiments at 10 to 12 days after eclosion from the puparia.

2.2. Methods

This study included six insecticide treatments tested against standard treatment (bait) mix, and one formulation (non-active) control treatment (Table 1), all tested with three leaf weathering times as detailed below. In addition, two other control treatments were tested with just one leaf weathering time; these were natflav (N) and keltrol (K) and water (W) and sugar (S). All treatments were made up immediately prior to use as 500 mL batches and mixed to ensure smooth homogeneous treatment solutions without lumps.

Table 1. Treatments included in this study.

Trt no.	Trt code	Insecticide	Insecticide a.i. concentration	Protein bait	Additive
1	FN	Fipronil ¹	0.005%	Natflav ²	-
2	FNK	Fipronil	0.005%	Natflav	Keltrol ³
3	SNK 1	Spinetoram ⁴	0.01%	Natflav	Keltrol
4	SN 2	Spinetoram	0.005%	Natflav	-
5	SNK 2	Spinetoram	0.005%	Natflav	Keltrol
6	SNK 3	Spinetoram	0.0024%	Natflav	Keltrol
8	NK	-	-	Natflav	Keltrol

¹ Maestro 200SC, ² Natflav® 500 (5%), ³ Keltrol (0.5%), ⁴ Success™ Neo 120SC.

Experiments were conducted in Beaudesert, south Queensland on adult fruit flies inside netting covered cages 30cm x 30cm x 30cm and allowed flies to be attracted and feed on insecticide bait mix weathered on foliage for 2 hours, 3 days and 6 days after treatment. After each weathering period leaf samples were placed on clean petri dishes in separate cages, where approximately 180 flies per cage had access to water and sugar only. The commencement of weathering was staggered so that all cages were set up simultaneously. The mortality in each cage and corresponding treatment was then recorded after 24 hours and then 72 hours.

Individual treatments were applied on target foliage by dipping them in treatment solution in a 15 ml beaker while still on the target tree (Figure 1). The leaf-dip method of insecticide application provides uniform coverage and used in bioefficacy studies with pest arthropods [8-11]. After dipping in treatment solutions, the leaves were allowed to age for 2 hours, 3 and 6 days in open weather conditions in full sunlight but were covered during rainfall events. The temperature during the experiments (February 2015) ranged from an average minimum 20°C to 32°C average maximum, while average humidity ranged from 50 to 90%.



Figure 2. Experimental setup in cages.

After the treated leaves were aged on the target trees for specific durations, they were carefully separated from the trees with scissors and cut into uniform sizes of 2cm x 5 cm. An individual treated leaf was placed in individual nylon cages of 30cm x 30cm x 30 cm size (Figure 2) with approximately 180 adult fruit flies in each cage. Throughout the duration of the experiment, the flies were provided with an unlimited supply of water and sugar cubes placed inside the cages. Each treatment was replicated two times approximately 1 month apart. Fruit fly mortality was counted after 24 hours and 72 hours exposure to the leaf sample. There were two sets of replicates completed a month apart. Each consisting of 23 treatments. Each within a randomized 3 block design.

2.3. Statistical Analysis

The statistical analysis for “% mortality” at each of 24 and 72 hours was carried out as an analysis of variance (anova) for a randomised complete block design, with the two blocks being the two-time replicates. This analysis involved just 18 of the treatments, with five treatments (the three NK treatments and the N and W/S treatments) being excluded since their data values were too close to zero, meaning they were likely to have lower variability than the other treatments, which would violate the anova assumption of homogeneity of variance. The 18 treatments had a 6 (insecticides) x 3 (leaf weathering times) factorial structure, and contrasts of interest were included in

the anova for each of the two factors. For the insecticide factor, the contrasts were: (a) linear trend in SNK rates, (b) quadratic curvature in SNK rates, (c) the effect of keltrol added to FN (FNK – FN), (d) comparison of FNK and SNK at their label rates (SNK2 – FNK) and (e) the effect of Keltrol added to SN at its label rate (SNK2 – SN2). For the leaf weathering time factor, linear and quadratic polynomial contrasts were specified. Following the anova, any pairwise comparisons of treatment means of interest were carried out using the unrestricted least significant difference (LSD) procedure [12].

3. Results and Discussion

Fruit flies continue to pose a significant threat to New Zealand horticulture. Since 1990, fruit flies have been detected on 12 occasions in traps laid under the MPI fruit Fly surveillance programme, which are strategically placed in high risk areas (Table 2). Two of those resulted in a full-scale eradication response by MPI leading to successful eradication from the incursion areas. In six out of ten incidents (60%), QFF (*Bactrocera tryoni*) was detected in the surveillance traps, a species known to cause significant losses in fruit production in Australia, New Caledonia and Tahiti [13]. It is a highly polyphagous species and attacks a number of fruit and vegetable crops including 60 wild hosts. This helps them build reservoirs for population resurrection to infest cultivated hosts [14-17].

Table 2. History of fruit fly detections in New Zealand.

Species	Location	Date	Outcome
<i>Bactrocera passiflorae</i>	Auckland	March 1990	Increased surveillance, no further finds
<i>Bactrocera tryoni</i>	Whangarei	May 1995	Increased surveillance, no further finds
<i>Bactrocera tryoni</i>	Auckland, (North Shore)	April 1996	Increased surveillance, no further finds
<i>Bactrocera papayae</i>	Auckland (Mt. Eden)	April 1996	Increased surveillance, no further finds
<i>Ceratitidis capitata</i>	Auckland, (Mt. Roskill)	May 1996	Eradication response with success
<i>Bactrocera tryoni</i>	Auckland, (Mt Roskill)	May 2012	Increased surveillance, no further finds
<i>Bactrocera tryoni</i>	Whangarei	January 2014	Increased surveillance, no further finds
<i>Bactrocera tryoni</i>	Whangarei	April 2014	Increased surveillance, no further finds
<i>Bactrocera tryoni</i>	Auckland, (Grey Lynn)	February 2015	Eradication response with success
<i>Bactrocera tau</i>	Auckland, (Manurewa)	January 2016	Increased surveillance, no further finds
<i>Bactrocera tryoni</i>	Auckland (Northcote)	February 2019	Increased surveillance, prophylactic baiting no further finds
<i>Bactrocera facialis</i>	Auckland (Otara)	March 2019	Increased surveillance, No further finds.

Experiments were conducted to compare the relative efficacy of fipronil and spinetoram and to determine if keltrol gel addition to bait mixes can improve the biological performance of the insecticides. The effect of weathering (of bait mix on target foliage) over time was also studied in the same experiments to determine the residual effectiveness of applied baits. This is an important aspect from an operational point of view as bait mixes that are biologically active over 3-7 days of time will require less frequent applications, thereby saving operational costs and increasing social acceptance of visiting operators in urban backyards.

3.1. Bioefficacy Enhancement of Individual Insecticides by Keltrol Gel

The addition of keltrol to both the fipronil and

spinetoram bait combinations significantly increased the mortality of caged flies ($p < 0.001$) for both the 24 hour and 3-day assessments (Tables 3a, 4 and 5). Keltrol (xanthan gum) is a heteropolysaccharide produced by fermentation using the bacterium *Xanthomonas campestris*. The primary structure of the molecule is composed of a backbone of 1,4-linked β -D-glucose with side chains containing two mannose and one glucuronic acids. Like sugar it is likely that keltrol acted as a phagostimulate encouraging the ingestion of the protein bait laced with insecticide. Similar results were found in another study in 2004 where keltrol enhanced the action of the insecticide bait resulting in significantly increased mortality [18].

The exact mechanism(s) on how the protein bait (natflav) and xanthan gum-based gel (keltrol) enhanced the application performance of insecticides is not scientifically studied. It is

hypothesised that a change in physico-chemical properties by mixing test insecticides with natflav and keltrol is responsible for enhanced retention on target surfaces and adequate uptake of oral dose by fruit flies. Another study was commissioned to study the physico-chemical properties of the different bait mixes comprising natflav and keltrol to determine formulation retention, rainfastness, surface tension, humectant (moisture retaining) properties and chemical degradation of insecticides (fipronil and spinetoram). Results from these experiments will be published in a follow up article in due course.

Although the flies aggregated on the leaf surface coated with the insecticide and protein bait (natflav) combination, these combinations returned relatively poor mortality results in this experiment in the absence of keltrol (Table 3a). At this stage it is difficult to determine why there was such a poor uptake from protein starved flies, which has reflected in the less than optimal mortality of these combinations. Had a comparable number of flies ingested these mixes, as with the keltrol results, then we would expect to have similar mortality rates. The level of mortality observed from these combinations would not be considered satisfactory in the field conditions during a response.

Table 3. Mean mortality (%) of *Bactrocera tryoni* after 24 and 72 hours of exposure to weathered treated leaves inside a cage. Main effect means for (a) the insecticide factor and (b) the leaf weathering time factor.

(a) Insecticide

	% mortality at:	
	24 hours	72 hours
FN	12.0	22.2
FNK	77.7	92.0
SNK1 (twice label)	82.8	97.4
SNK2 (label rate)	69.1	95.2
SNK3 (half label)	45.5	90.0
(NK)	(0.6)	(1.6)
SN2 (label rate)	4.2	19.4
LSD (5%)	13.0	8.6
<i>Significance of contrasts:</i>		
SNK rates, linear trend	***	ns
SNK rates, quadratic curvature	ns	ns
FNK vs FN	***	***
SNK2 (label) vs FNK	ns	ns
SNK2 (label) vs SN2	***	***

(b) Leaf weathering time

	% mortality at:	
	24 hours	72 hours
2 hours	62.1	76.4
3 days	48.2	67.8
6 days	35.4	63.9
LSD (5%)	9.2	6.0
<i>Significance of polynomial contrasts:</i>		
Linear trend	***	***
Quadratic curvature	ns	ns

Note: ns=not significant; ***=0.1% significant.

The improved bait mix formulated using keltrol was visibly viscous and could be effectively applied using a hand-held drench gun. This reduced the drift and the previously experienced run-off to the ground, thereby allowing

maximum delivery and adhesion on the target leaf surfaces. It also provided a good opportunity to use half the volume (50 ml instead of 100 ml) while substantially improving bioefficacy (as compared to using no keltrol) and providing bonus operational safety and economy in fruit fly baiting programme.

Within the first 24 hours, the experiment also showed that freshly dipped citrus leaves that had only 2 hours weathering resulted in the highest mortality of the caged flies (Tables 3b and 4). A significant decline with increasing weathering time was noticeable on all combinations (FNK, SNK2 and SNK3) except with SNK1 (twice label rate) (Table 4). The mortality of the caged flies continued to increase after 24 hours for all insecticides (Tables 3a and 5). After 3 days, over 90% of flies had succumbed to the SNK1 and SNK2 insecticide bait and keltrol combinations for all leaf weathering times (Table 5).

3.2. Spinetoram Dose Effects

There was a highly significant linear trend in the main effect means of spinetoram dose when the mortality of the caged flies was assessed at 24 hours ($p < 0.001$), with percentage mortality increasing from 45.5% at the half label rate to 82.8% at the twice label rate (Table 3a). When the mortality assessment was redone at 72 hours the trend was still apparent but not statistically significant, with percentage mortality increasing from 90.0% at the half label rate to 97.4% at the twice label rate (Table 3a).

When the 24 hour mortality results were examined for each leaf weathering time separately, the linear trend of increasing mortality with increasing spinetoram dose rate was highly significant for both 3 and 6 days of weathering on the leaf, but not significant for 2 hours of leaf weathering (Table 4). For the 3-day mortality results, the linear trend of increasing mortality with increasing spinetoram dose rate was not statistically significant for either the 2 hours, 3 days or 6 days leaf weathering times (Table 5).

Table 4. Mortality (%) of *Bactrocera tryoni* from different treatments after 24 hours of exposure to treated leaf weathered for 2 hours, 3 days and 6 days.

Insecticide	Leaf weathering time		
	2 hours	3 days	6 days
FN	15.4	12.4	8.2
FNK	91.2	83.0	59.1
SNK1 (twice label)	94.0	78.1	76.1
SNK2 (label rate)	86.0	73.0	48.4
SNK3 (half label)	78.0	39.3	19.1
(NK)	(0.0)	(1.2)	(0.5)
SN2 (label rate)	7.9	3.5	1.2
(N)	-	-	(2.1)
(W/S)	-	-	(1.3)
LSD (5%)		22.6	
<i>Significant interaction contrasts:</i>			
(SNK rates, linear trend) x (Weathering time, linear trend)			
5% significant			

3.3. Effect of Weathering Time of Baits on Bioefficacy

There was a highly significant linear trend in the main effect means of leaf weathering time when the mortality of the caged flies was assessed at 24 hours ($p < 0.001$), with cumulative average percentage mortality decreasing from 62.1 for 2 hours weathering to 35.4 for 6 days weathering (Table 3b). Similarly, there was a highly significant but less marked linear trend in the main effect means of weathering time when the mortality of the caged flies was assessed at 3 days ($p < 0.001$), with percentage mortality decreasing from 76.4% for 2 hours weathering to 63.9% for 6 days weathering (Table 3b).

When the 24-hour mortality results were examined for each insecticide separately, the linear trend of decreasing mortality with increasing weathering time was statistically significant for FNK, SNK2 and SNK3 (Table 4). For the 3-day mortality results, the linear trend of decreasing mortality with increasing weathering time was statistically significant for only one insecticide, SN2 (Table 5).

Table 5. Mortality (%) of *Bactrocera tryoni* from different treatments after 3 days of exposure to treated leaf weathered for 2 hours, 3 days and 6 days.

Insecticide	Leaf weathering time		
	2 hours	3 days	6 days
FN	27.7	23.0	16.1
FNK	96.0	91.5	88.3
SNK1 (twice label)	99.1	94.2	98.8
SNK2 (label rate)	97.8	97.1	90.9
SNK3 (half label)	98.6	86.9	84.4
(NK)	(0.9)	(1.4)	(2.5)
SN2 (label rate)	39.3	14.2	4.6
(N)	-	-	(2.6)
(W/S)	-	-	(1.6)
LSD (5%)		14.8	
Significant interaction contrasts: (SNK2 versus SN2) x (Weathering time, linear trend) 5% significant			

The tests showed that after 3 days' weathering, leaves dipped with insecticide combinations showed a high level of mortality after 3 days of exposure (Table 5). Leaves that had been weathered for 6 days showed a small but consistent drop off in mortality after 3 days of exposure (Table 5). The one exception was spinetoram at twice the label rate (0.01% ai); increased from 94.2% mortality at 3 days leaf exposure to 98.8 at 6 days.

4. Conclusions

Keltrol (xanthan gum) gel substantially enhanced bioefficacy of fipronil and spinetoram insecticides. It should be considered an integral component of protein bait mixes for spot applications in a fruit fly eradication response. The improved protein bait mixes delivered an enhanced insecticide dose uptake via oral route that resulted in significantly increased mortality rates of the caged flies.

At a 24-hour mortality check FNK (fipronil / natflav / keltrol), SNK1 (spinetoram twice the label rate / natflav /

keltrol) and SNK2 (spinetoram at label rate) had performed significantly better than FN or SNK3 (spinetoram half the label rate) on treated leaves that had been weathered for 6 days. However, the mortality data at 3-days was tighter and there was no significant difference between FNK, SNK1, SNK2 or SNK3, though the latter also had the lowest mortality.

This study showed that spinetoram could be a useful alternative to fipronil providing it is used at 0.01% ai, which is twice the label rate. At a 3-day count of dead flies, there was no statistically significant difference between the label rate of spinetoram at 95.2% compared to fipronil at 92.0% mortality.

Flies feeding of leaf surfaces weathered for 3 days recorded a drop in mortality but this was not statistically significant. However, all but SNK1 showed a diminishing mortality trend towards 6 days of leaf weathering. This trend was the least significant with FNK. Based on the results, SNKI (spinetoram at twice the label rate) appears to be a worthy alternative insecticide to fipronil. Although rain would necessitate re-baiting in the field, this experiment showed that keltrol-mediated enhanced bait containing either fipronil or spinetoram could remain effective for up to 6 days.

Addition of keltrol not only improved bioefficacy but also improved the viscosity of the mixture thereby reducing its drift potential and applicability using spot spray guns for better public acceptability, safety and economy.

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