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SPINOSAD APPLICATION PREVENTS DAMAGE BY *AGRIOTES* SPP. LARVAE (WIREWORMS) AND PROTECTS MAIZE (*ZEA MAYS*) YIELD IN NORTHEAST ITALY

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ABSTRACT

Agriotes spp. larvae, commonly known as wireworms, are major pests that cause great economic damage to many European crops. To combat them, most farmers prophylactically apply soil insecticides, including high-impact ones such as neonicotinoids. However, due to their hidden life cycle below ground, wireworms are difficult to control, especially in organic farming where persistent, non-specific soil insecticides cannot be used. As legislation tightens regulation on chemical insecticides that have harmful effects on humans and the environment (such as the withdrawal of many chemicals and the general limitation of all of them), biological control agents are gaining attention as an alternative strategy. The aim of this study was to investigate the agronomic effectiveness of Spinosad, a bioinsecticide, in row application during planting to manage wireworms in maize (*Zea mays*) in Northern Italy. Based on the performance of Spinosad, even in harsh conditions as observed throughout this study, and its ability to mitigate yield losses compared to the non-treated control, we conclude that this product can be used as part of an Integrated Pest Management to mitigate the damages caused by this pest in organic agriculture. However, further studies are required to better understand how this bioinsecticide can be integrated into *Agriotes* spp management to reduce economic losses and have a positive impact on the environment and public health.

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INTRODUCTION

Wireworms are the larvae of click beetles (Coleoptera: Elateridae). They consist of more than 9,000 species distributed worldwide and some are important pests of a wide variety of crops such as potato, carrot, sugar beet, sugarcane, and maize. Barsics et al. (2013) utilizing different sampling and identification methodologies e.g., pheromone traps and molecular identification of collected larvae, placed throughout Europe allowed a reliable mapping of species, showing that damages due to wireworm infestation are mainly attributed to the genus *Agriotes*.

The main damages of wireworms feeding occur on neck and belowground organs. These insects feed on the roots of the seedlings causing lodging, mortality, yield losses (Reddy et al., 2014; Furlan et al., 2017), and resulting in significant economic issues across several European countries (Benjamin et al., 2018). Due to their below-ground life cycle, overlapping generations, polyphagous nature, and their adaption to a wide range of agricultural ecosystems (Furlan, 1998, 2005; Ritter and Richter, 2013; Sufyan et al., 2014; Traugott et al., 2013, 2015), wireworms are difficult to control, especially in organic farming, where persistent, non-specific soil insecticides cannot be used. As a result of these regulatory changes on the utilization of chemical pesticides, the need for biological products have increased (Schepl and Paffrath, 2007; Brandl et al., 2016; Benjamin et al., 2018).

However, it has been emphasized that the abandonment of ecotoxicologically problematic soil insecticides may increase wireworm-related problems (Parker and Howard, 2002; van Herk et al., 2008). This pest has been commonly controlled with synthetic organochlorines, organophosphates, carbamates, neonicotinoids, pyrethroids and phenyl pyrazole (Kuhar et al., 2003; Barsics et al., 2013). Although these insecticidal treatments are efficacious but due to concerns about human and environmental health, there is increasing interest in developing and using low-risk compounds for insecticide treatments.

In this context, Spinosad, a low-risk insecticide derived by fermentation from the soil actinomycete, *Saccharopolyspora spinosa* has become a unique pesticide with a high selective activity against targeted pests and low toxicity in non-target organisms (including many beneficial arthropods) (Bourdon et al., 2021; Ericsson et al., 2007). These characteristics make Spinosad an excellent new tool for integrated pest management (Guojun et al., 2016, Racke et al., 2006). Currently, the pesticide has been registered in several countries as seed treatment at a maximum rate of 1 ppm (1 mg a.i. kg^{-1} of seeds) and with the Maximum Residue Level (MRL) or tolerance on grains set at 1 or 1.5 ppm (Hertlein et al., 2011). The insecticide is highly active by both contact and ingestion to numerous pests in the orders Lepidoptera, Diptera, Thysanoptera, Coleoptera, Orthoptera, Hymenoptera, and others (Bret, 1997). It affects nicotinic acetylcholine and gamma amino butyric acid (GABA) receptors' sites of the insect nervous system, and so far has proved non-cross-resistant to any other known insecticide (Salgado and Sparks, 2005). In addition, Spinosad exhibits low mammalian toxicity and a highly favourable environmental profile (Cleveland et al., 2001, Nikoukar and Rashed, 2022). Spinosad is considered as a bioinsecticide and thus has been approved for use in organic agriculture by many national and international certification bodies (Racke, 2006; Mandour, 2009). Additionally, previous research has reported the effectiveness of Spinosad in controlling several pests in many crops (Mandour, 2009; Dissanayaka et al., 2020).

The aim of the present study was to document the effectiveness of Spinosad in row application during planting to manage *Agriotes* spp. larvae in maize (*Zea mays*) in Northern Italy. Based on our investigation of Spinosad application, we report that this product can be used as part of an Integrated Pest Management (IPM) to mitigate the damages and losses caused by this pest in organic agriculture.

MATERIALS AND METHODS

Experimental design

The experiment was carried out in Friuli Venezia Giulia region (Italy, NE), in 2018. A commercial maize field, hybrid DKC 5911 with a population of 70,000 seeds ha^{-1}; and 75 cm inter-row width. The field fertilization was performed @ 240 kg of N. ha^{-1} and pre-emergence plus post-emergence herbicide treatments causing very low weed densities. Two treatments were tested as follows: non-treated control (NTC), and Spinosad directly in the row during planting, applied using a commercial sprayer following the label recommendation of 25 $mL.L^{-1}$, thus, 120 L of solution per hectare, properly weighted and pondered for our experimental blocks. Spinosad was bought (Dow agrochemicals, USA), thus, it is a commercial product being used in the study-area for many a purposes. The experimental units' "plots" were consisted of 8 rows of 5 meters spacing 0.75 meter between them. The experimental design was RCBD (Randomized Complete Block Design) with four replications. The soil in the experimental area is characterized as Hypercalcaric Regosol (Humic) and the weather in classified accordingly to Koppen id "Cfb" (Marine West Coast Climate). The Figure 1 shows the weather conditions observed throughout 2018.

Effectiveness assessment

The effectiveness of Spinosad for controlling wireworms was assessed through the evaluation of the percentage of germinated plants, percentage of damaged plants in two different growth stages: germination V3 (three leaves totally expanded, with growing point below-ground) and V6 (six leaves totally expanded, with the growing point above-ground), number of *Agriotes* spp. larvae before harvest and grain yield (YD).

Percentage of emerged plants and damage

The percentage of emerged plants (stand) was determined in the four centre rows at V3. In these segments, all emerged seedlings were also categorized in two groups: wireworm-damaged (e.g., leaves exhibiting drilling holes, dead central leaf, yellow stripes on leaves) and undamaged (plants with normal development). Since plants with mild symptoms (e.g., leaves exhibiting drilling holes or yellow stripes on leaves) can recover from previous attack in their early growth stages, the damage assessment was recorded twice at the growth stages V3 and V6.

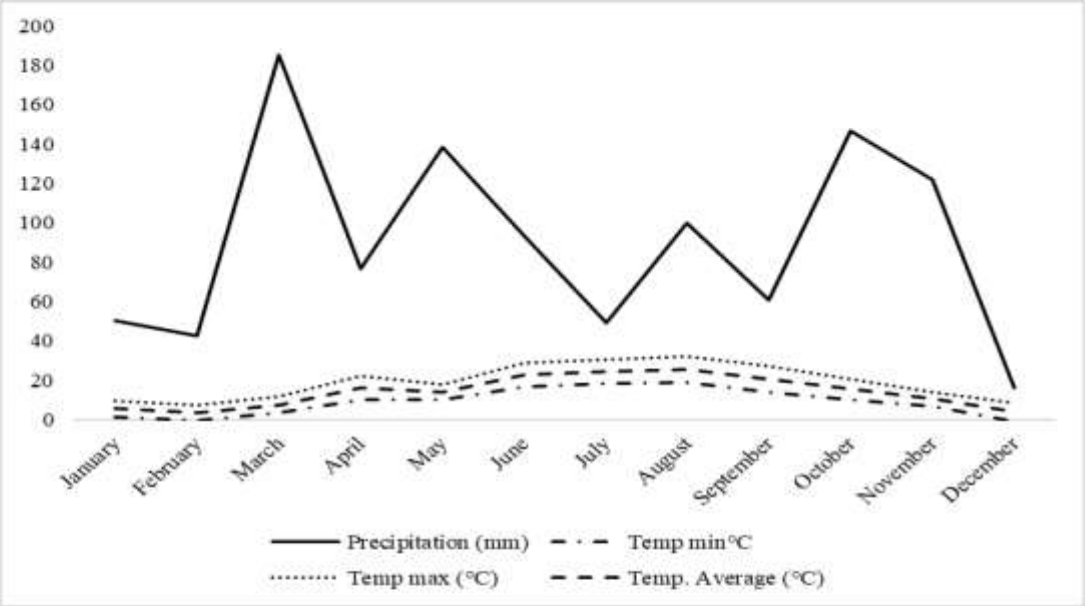

Figure 1. Precipitation (mm), minimum and maximum temperature (°C) in Pordenone, Friuli Venezia Giulia region (NE, Italy) in 2018.

Number of wireworms before harvest

Seed baits have been extensively examined for their utility in determining wireworm populations (Simmons et al., 1998). To gather information on the residual effect of Spinosad throughout the crop cycle, traps were installed 3 weeks before harvesting. The traps consisted of a 140 mL mixture of 1:1 untreated maize/wheat seed (soaked in water 24 h before placement to facilitate germination) placed in a hole (10 cm wide by 25 cm deep) and covered with soil. In order to collect solar radiation to warm the soil and facilitated germination, a black polyethylene trash bag (45 by 38 cm) was placed on the soil surface to prevent disturbing the baits, and to avoid displacement by wind, the edges of the trash bags were covered with soil. Eight traps (2 in each block) were placed in each block. All traps were collected a week prior to harvesting time. The collection of a sampling units included removal of the maize and wheat seeds and seedlings, as well as the soil surrounding the bait (approximately 1500 mL).

Yield measurement

Total yield was recorded, expressed as kg.ha^{-1}. Harvest was done by using a plot-harvester. Four centre rows were harvested (15 m^{2}) and grain weight per plot was expressed in tons.ha^{-1} at 15% of moisture.

Statistical analysis

One-way analysis of variance was performed by using "R" free software (version 4.0.3 2020-10-10). Statistical analysis to determine significant differences between treatment means was carried out using the Tukey SD test ($p \leq 0.05$), the data are the average of 4 replicates (Mian et al., 2022).

RESULTS

The average amount of precipitation (1081.6 mm), minimum (9.1°C) and maximum (19.3°C) temperatures observed in the research site throughout the experimental period are shown in Figure 1. May was the month with higher amount of precipitation on average, 138.2 mm. On the other hand, the month with the least precipitation was July with an average of 49.5 mm. In terms of liquid precipitation, the month with the highest number of raining days was May (20 days) and the least number of raining days was in August (8 days). The temperatures ranged between 10°C and 32°C in the months of May and August, respectively. These conditions were between the optimum range for normal growth and development of maize in the region.

As observed during the months of June, July, and August, the average temperature ranged between 22.6 and 25.4°C.

The percentage of emerged plants in response to the treatments is shown in Figure 2. The in-row application of Spinosad (25 mL.L^{-1}) during planting, protected the seedlings in their earlier growth stages. Consequently, the plots treated with Spinosad showed higher percentage of emerged plants per linear meter (stand). As presented in Figure 2, Spinosad application was responsible for 91% of emerged plants, this value being 9 % higher than the NTC.

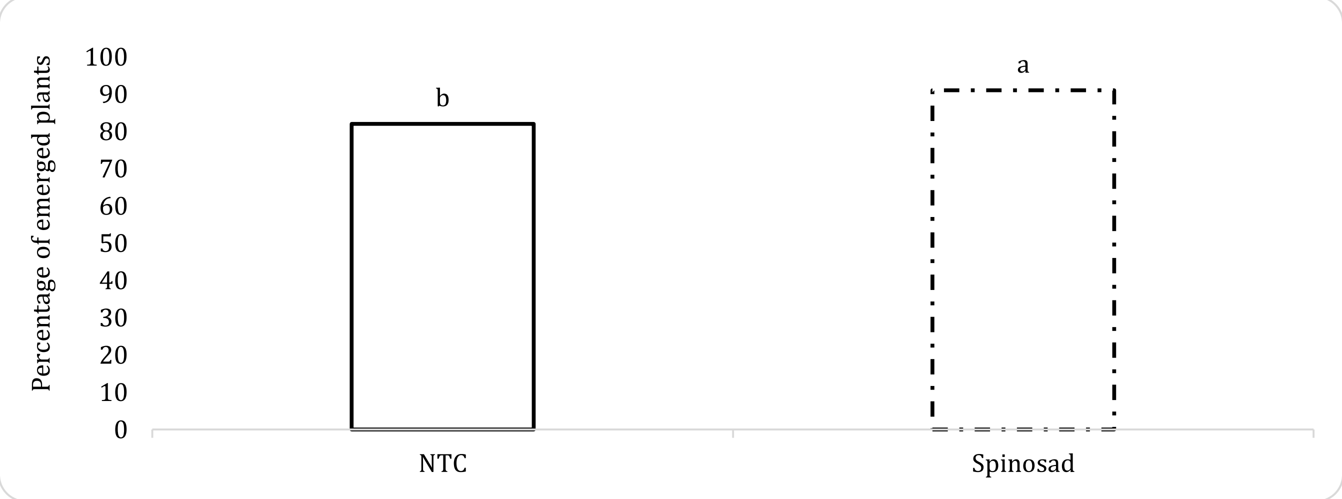

Figure 2. Effect of Spinosad in-row application during planting on the percentage of emerged plants in maize hybrid DKC 5911. Means assigned by different letters are statistically different accordingly to the Tukey Test ($p \leq 0.05$). NTC: non-treated control.

The Figures 3A and B show the percentages of damaged plants in the V3 and V6 growth stages respectively. In the first evaluation carried out at V3, the NTC the percentage of wireworm-damaged plants was 51.6% higher than observed for the Spinosad treatment (Figure 3A). In the following evaluation (V6), a damage reduction was observed in both treatments. However, even with this reduction, plants treated with Spinosad showed 57% less damage than the NTC (Figure 3B).

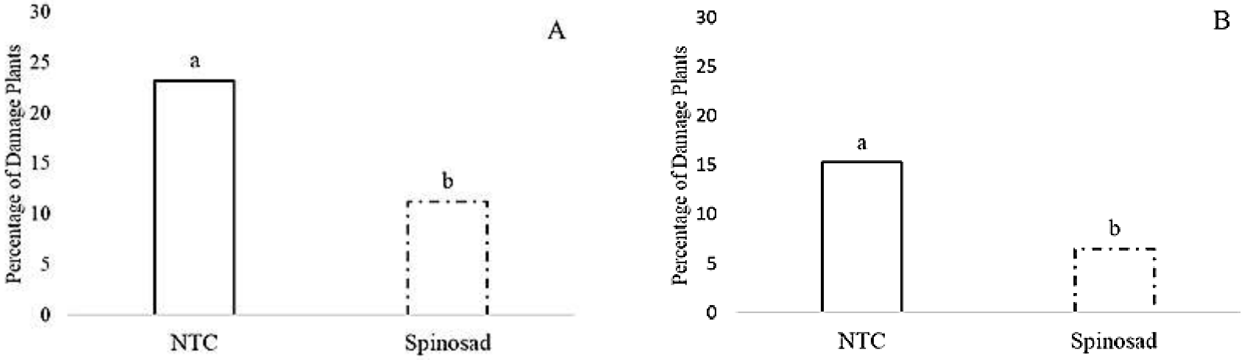

Figure 3. Effect of Spinosad in-row application during planting on percentage of damaged plants by *Agriotes* sp at A and B growth stages in maize hybrid DKC 5911. Means assigned by different letters are statistically different accordingly to the Tukey Test ($p \leq 0.05$). NTC: non-treated control.

To have insights on the *Agriotes* spp. population, an assessment of the number of larvae was done before harvest. In this evaluation, the number of larvae in the NTC plots was 23.9% higher than the Spinosad, the values observed were 123.5 and 94% respectively (Figure 4).

The necessity of an integrated management of this pest is reinforced by the reduction of 2.59 tons (43 bags of 60 kg) in the NTC plots, this value being 22.7% in comparison with the Spinosad-treated plots (Figure 5).

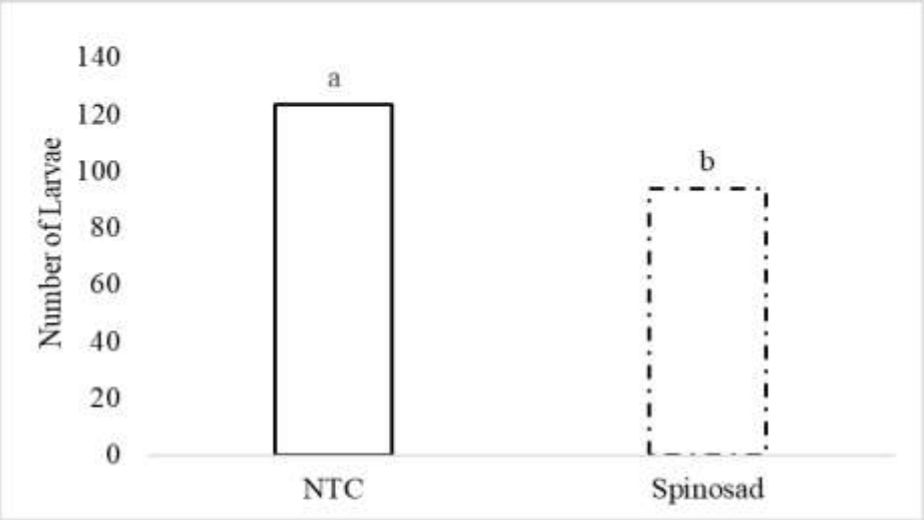

Figure 4. Effect of Spinosad applied in-row on the number of *Agriotes* larvae prior to harvest in maize hybrid DKC 5911. Means assigned by different letters are statistically different accordingly to the Tukey Test ($p \leq 0.05$). NTC: non-treated control.

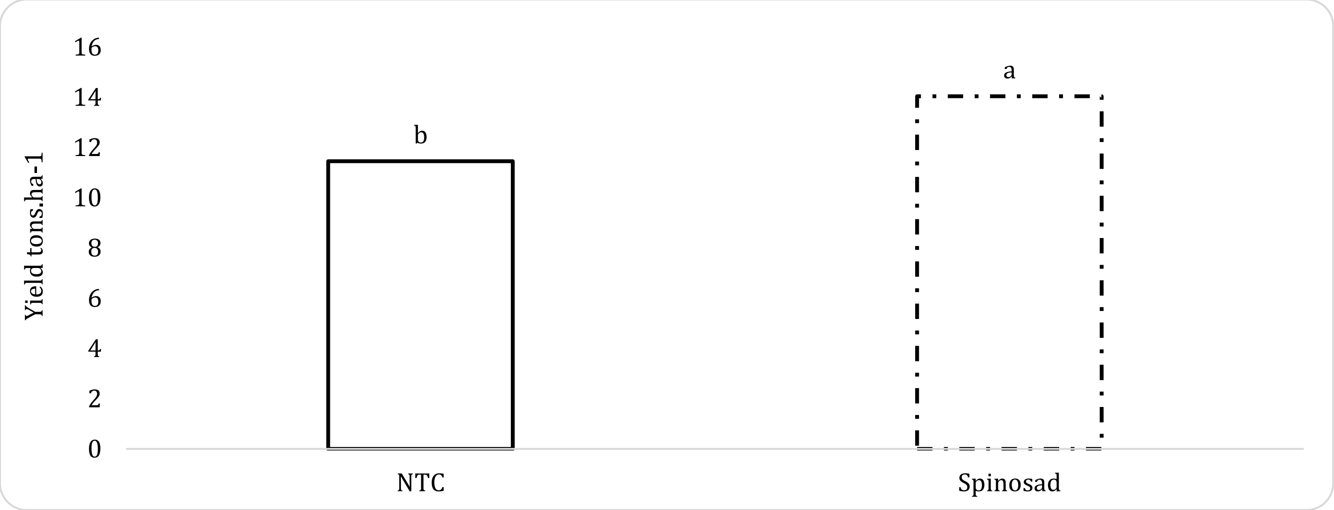

Figure 5. Effect of Spinosad applied in-row on yield (tons.ha^{-1}) in maize hybrid DKC 5911. Means assigned by different letters are statistically different accordingly to the Tukey Test ($p \leq 0.05$). NTC: non-treated control.

DISCUSSION

The identification of biological insecticides and their effectiveness on IPM strategies against wireworms has been extremely difficult due to the lack of available information on the key aspects of concerned species (Furlan, 2015, Veres et al., 2020). Also, there is lack of information on alternative products such as Spinosad in field conditions (Salgado et al., 2005, 2010). To date IPM strategies have increased, however have not played yet a significant role in maize and other arable crops (Furlan and Kreutzweiser, 2015) despite the strong negative impact of using soil insecticides (i.e., neonicotinoids) to control wireworms (van der Sluijs et al., 2015).

Maize is very vulnerable to wireworm damage from sowing until the 10/12-leaves stage (V10/V12). Seedlings attacked by this pest usually die before they reach the V6 stage, which can be explained by the fact that until V5 (five expanded leaves); the growing points' attacks. After the 8-leaves stage (V8), the plant was much less vulnerable; however attacks still can induce the reduction of ears. Significant differences in stand were observed between treatments, during both assessments. This result is aligned with our findings and what was reported by Poggi et al. (2021), suggesting that Spinosad application reduced the wireworm's population and the damage leading to a higher stand.

To start off with, as reported by Furlan et al. (2017) rainfall and temperatures did not influence risk particularly, although temperatures above 16°C can increased the damage probability. Higher temperature may allow larvae to moult more quickly and then increase the number of larvae in a potentially harmful feeding phase (Furlan 1998, 2005). Hence, our conditions were the best for this type of trial.

Going more in detail, damaged assessments done at V3 and V6 once plants have completely emerged. At V3, it was recorded 11.25% of symptomatic plants in Spinosad-treated plots, whilst 23.5% in NTC. Hence, we can attribute the lower damage due to the Spinosad performance reducing the wireworm population. This trend was also confirmed by the survey conducted at V6. In this evaluation only 6.5% of Spinosad-treated plants showed damage symptoms, while in NTC, 15.25% of plants were damaged.

The Figure 4 showed the impact of Spinosad on the cumulative number of larvae (pest density) before harvest in response to Spinosad application in the beginning of the season. Using specific traps, we have been able to collect and record the number of wireworms present in each treatment. In fact, NTC counted, on average, 123.5 wireworms, whilst Spinosad-treated plots 94. These results confirm that the selected study-site is historically highly infested by wireworms, evidencing that Spinosad is a promising tool to be used in the Integrated Pest Management in organic farming (Vernon et al., 2013). The higher stand and the reduction in damaged as mentioned above translated into 22.7% yield protection in the Spinosad-treated plots with low impact to the environment and human health.

So far, Spinosad resulted being a good tool to combat *Agriotes* sp. in maize. Of course, it could have different benefits towards other pathogens in others crops (Parker et al., 2002). Finally, Spinosad being a natural product as above-mentioned, the environmental impact is really low, also in a long-term application strategy for IPM, as reported by Michaelakis et al. (2020). Last but not least, the cost for farmers is low if compared to agrochemicals: Spinosad costs on average 36$/L, indeed other chemicals about 130$/L.

CONCLUSIONS

To the best of our knowledge, this is the first study where Spinosad was applied in row during the planting, having positive effect on stand, reducing plants damage and *Agriotes* sp. population reduction, leading to yield protection in maize in Northeast Italy. Our approach clearly indicated its effectiveness, not only in terms of plant health status, but final yield as well, together with applying an environmentally-friendly product. Further studies are required to better understand how this bioinsecticide can be integrated to *Agriotes* sp. management for organic agriculture, reducing the economic loses, leading to a positive impact on the environment and public health. Not only, could Spinosad be used in several agricultural chains, not only for wireworms control in maize.

AUTHORS' CONTRIBUTIONS

GM conceptualized, designed experimental set up and collected the data, GM and FC analysed the data statistically, wrote the manuscript, supervised the research, proofread and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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