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#### **Research Article**

# Aryloxyphenoxypropionates tolerant and non-tolerant corn: plant-back interval after acetyl-coA-carboxylase inhibitors applications

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#### Conflict of Interest:

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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#### **HIGHLIGHTS**

- Aryloxyohenoxypropionates require higher intervals than cyclohexanediones to corn sowing.
- The application of haloxyfop-ethyl prior sowing of KWS9110 and 2B578 hybrids must be earlier than 8 and 11 days, respectively.
- Enlist corn hybrids can be safely sown immediately after ACCase applications.

#### **ABSTRACT**

**Background:** The application of Acetyl CoA carboxylase (ACCase) inhibitors prior to sowing of grass crops may result in crop damage due to residual effect of these herbicides in soil. The hypotheses was that different corn hybrids and ACCase herbicides can result in different plantback intervals after burndown treatments.

**Objective:** This work was planned to determine safe plant-back periods for three corn hybrids where ACCase herbicides clethodim and haloxyfop-methyl are applied.

**Methods:** Herbicides were sprayed at 21, 14, 7, 2 and 0 days before sowing (DBS) at 192 g a.i. ha<sup>-1</sup> (clethodim) and 120 g a.i. ha<sup>-1</sup> (haloxyfop-methyl). Individual experiments were carried out with three hybrids: KWS9110 and 2B587 with glyphosate and glufosinate tolerance and Enlist with aryloxyphenoxypropionate tolerance.

**Results:** Haloxyfop-methyl resulted in higher damage potential compared to clethodim for the hybrids KWS9110 and 2B587. For these hybrids, significant crop stand and grain yield reductions were observed mainly when haloxyfop was applied closer to sowing date (7, 2 and 0 DBS).

**Conclusions:** Based on the effects on corn grain yield, a safe plant-back period for haloxyfop was estimated to be 8 and 11 days for the hybrids KWS9110 and 2B587, respectively. For the Enlist corn hybrid, there were no yield decreases, even when haloxyfop was applied at 0 DBS. Clethodim did not affect corn yield of any hybrid in any application time.

#### **1** INTRODUCTION

In addition to glufosinate- and glyphosate- tolerance, Enlist corn hybrids carry the aryloxyalkanoate dioxygenase trait (AAD-1), obtained from the bacteria *Sphingobium herbicidovorans* (Ruen et al., 2017). The gene codifies enzymes able to metabolize herbicides such as 2,4-D and aryloxyphenoxypropionates

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providing tolerance to these herbicides in corn hybrids (Ruen et al., 2017).

The Acetyl-CoA carboxylase (ACCase) inhibitors remain the most important group of herbicides for post-emergence grass control (Sanafar et al., 2017). These herbicides block the synthesis of fatty acids in monocots due to the inhibition of the target ACCase enzyme, leading to plant death (Burton et al., 1989). The two major chemical groups with this mechanism of action are the Aryloxyphenoxypropionates (FOP's) and Cyclohexanediones (DIM's), which are extensively applied to different crops such as soybeans, cotton, sunflowers and peanuts (Kukorelli et al., 2013). A third chemical group is the Phenylpyrazolines (DEN's), which includes the herbicide pinoxaden, that has been used mainly to provide grass control in winter cereals (Locke et al., 2002). The relevance of this mechanism of action has improved substantially in Brazil over the last decade, due to its efficiency in controlling glyphosate-resistant grasses such as tall windmill grass (Chloris elata), goosegrass (Eleusine indica), Italian ryegrass (Lolium perenne ssp. multiflorum) and sourgrass (Digitaria insularis).

Glyphosate resistant (GR) populations of sourgrass were first reporded in Brazil in 2008 and are currently widespread over all grain-producing areas of the country (Lopez Ovejero et al., 2017) and in neighboring countries of Argentina, Paraguay and Bolivia. Previous research reports confirmed efficient control of GR sourgrass can be achieved with ACCase inhibitors (Melo et al., 2012; Correia et al., 2015). Particularly for adult plants, the available options for efficient control on post-emergence condition are based on sequential applications of these herbicides, alone or associated to other mechanisms of action (Melo et al., 2012; Zobiole et al., 2016). In many field situations, prescribed doses of ACCase are much higher today after the spread of GR sourgrass. Prior to crops sowing, farmers have adopted as much as twice the labeled doses for haloxyfop or clethodim. Many of those areas are further sown with corn, wheat or other winter cereals. Problems associated to carryover of those herbicides to grass crops have been detected when the ACCase herbicides are applied next to the sowing date. Even though these chemicals are considered mainly as post-emergence herbicides, there is a chance they produce residual effect in soil, especially if higher doses are used (Mahoney et al., 2016; Lancaster et al., 2018).

This is an increasingly serious problem for those areas where corn is immediately sown after soybean

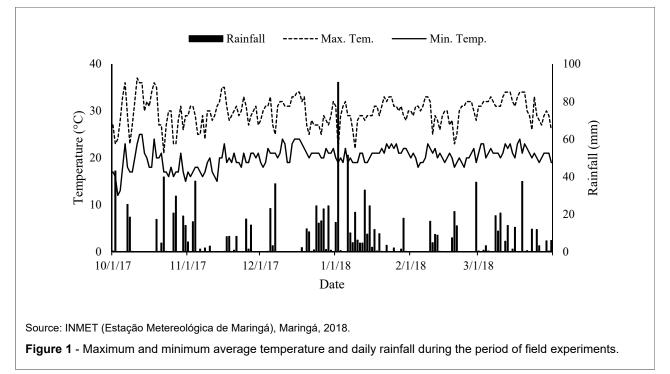
harvest, where clump escapes of sourgrass must be readily controlled before corn sowing. Usually, the interval between soybean harvest and corn sowing is as short as possible, since the delay in crop sowing leads to increased risks to yield related to inappropriate climatic conditions during the cycle (Garcia et al., 2018). Therefore, this research hypothesized that ACCase applications next to or at corn sowing date may be harmful to the growth, development and grain production of corn, based on eventual residual effect of these herbicides on soil.

Given the considerations above, this work was developed to determine safe plant-back timings for three corn hybrids after ACCase herbicides clethodim and haloxyfop-methyl applications.

#### **2 MATERIAL AND METHODS**

Three field experiments were carried out from 11/01/2017 to 04/04/2018, at Iguatemi Experimental Farm (Universidade Estadual de Maringá, Maringá – PR, Brazil). Chemical analysis of soil samples from the experimental site (0-20 cm depth) had the following composition:  $pH_{H_2O}$ : 6; Organic matter: 2.1%; CEC: 5.28 cmol<sub>o</sub> cm<sup>-3</sup>; 73.9% sand, 5.7% silt and 20.4% clay. Figure 1 illustrate the meteorological data during the period of the work. Soil from the experimental site was plowed with a harrow disc 25 days prior to the start of field experiments. Five days prior to corn sowing, glufosinate (600 g a.i. ha<sup>-1</sup>) was applied to the field as a burndown for eventually emerged weeds.

For each experiment (Exp.), increasing periods of time between the application of herbicides clethodim and haloxyfop-methyl and corn sowing were evaluated. Each experiment was comprised of one corn hybrid (Exp. 1 = KWS9110, Exp. 2 = 2B587, Exp. 3 = Enlist<sup>™</sup>). The hybrid KWS9110 was chosen based on previous work that it demonstrated visual injuries in areas where haloxyfop-methyl had been applied (data not published). The choice of hybrid 2B587 was based on its widespread use in different geographical areas of Brazil compared to KWS9110 (Dow AgroSciences, 2018) thus making hybrid. The last hybrid, Enlist corn, is one of the first corn hybrids available in Brazil and harboring haloxyfop-methyl tolerance. The experiments were conducted in a factorial scheme (5 x 2) + 1, including five periods of time between herbicide application and corn sowing (21, 14, 7, 2 and 0 days before sowing - DBS), two herbicides (clethodim at 192 g a.i. ha-1 and haloxyfopmethyl at 120 g a.i. ha<sup>-1</sup>), and an additional treatment represented by a non-sprayed check. Doses of



clethodim and haloxyfop-methyl were based on recommendations considered as enough to provide effective control of adult plants of sourgrass at burndown prior to sowing (Zobiole et al., 2016).

Climatic conditions at each application are presented in Table 1. All applications were made with a  $CO_2$ -pressurized backpack sprayer, equipped with a 2-m-long bar containing four ST-0.15 spraying nozzles, with a pressure of 207 kPa and speed of 1 m s<sup>-1</sup>, providing a spray volume equivalent to 150 L ha<sup>-1</sup>.

 Table 1 - Application dates and climatic conditions at herbicide application. Maringá, 2018

Application (DBS)	Date	Relative air humidity (%)	Wind speed (km h <sup>-1</sup> )	Temperature (°C)
21	11/01/2017	70	1.0	24
14	10/08/2017	65	1.2	25
7	10/15/2015	71	2.6	25
2	11/20/2017	60	3.0	26
0 (apply and plant)	11/22/2017	62	2.0	28
DBS: days before sowing				

DBS: days before sowing.

The plots were composed of three rows (0.9 m) between rows) that were 5 m in length  $(13.5 \text{ m}^2)$ . Ratings for crop tolerance were taken on the center row excluding the 0.5 at both ends. A randomized block experimental design was used, with four replicates per treatment.

Herbicides were applied at different dates before crop sowing. All experiments (hybrids) were sown on the same day (11/22/2017 - 0 DBS), with application of 400 kg ha<sup>-1</sup> of NPK 11-18-14 fertilizer. Sowing density was 6.5 seeds m<sup>-1</sup>. When corn plans reached

V3 stage, a complementary nitrogen topdressing application (urea, 60 kg ha<sup>-1</sup>) was applied. To provide control of dicot weeds, a layby application of atrazine (1.5 kg ha<sup>-1</sup>) was carried out when the corn was on V3 stage. All experiments were hand-weeded weekly during the crop cycle.

Corn injuries caused by the herbicides were evaluated visually at 5, 14, and 21 days after emergence (DAE), based on a 0% to 100% scale, wherein 0% is equivalent to no damage, and 100% is equivalent to the death of all plants (SBCPD, 1995). Crop stand and plant height were evaluated at 5 and 21 DAE, by counting or measuring (from soil to the collar of the last fully expanded leave) all plants in the central 4m of central row in each parcel

To determine the corn grain yield of each treatment, all cobs from the useful area were manually harvested (04/04/2018). The cobs collected were threshed with the help of a manual thresher, and the grains were separated from the impurities. Samples were taken to determine the moisture (portable moisture determiner model Mini GAC) of each plot, and the yield data was converted to 14% of moisture.

Data were subjected to analysis of variance by F-test (p<0.05), and when interactions were significant, the levels of factor herbicides were unfolded, and means were compared by F-test (p<0.05).

For the factor periods of time between herbicide application and corn sowing, a regression analysis was performed for each significant variable for crop stand and yield. For crop stand and corn yield, a nonSBCPD | Planta Daninha

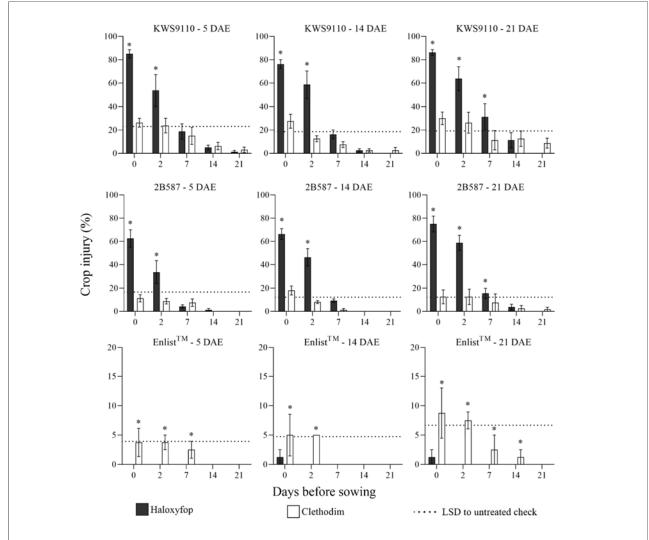
linear model with an exponential function  $\hat{Y} = c + a(1 - exp^{-bx})$  was fitted. For crop yield, considering the maximum grain yield estimated by the model, the number of days between herbicide spraying and corn sowing that would result in a maximum decrease of 2% in grain yield was calculated, considering this amount as an acceptable loss (Melo et al., 2001). When a significant contrast was found between additional checks and other treatments, the means were compared by Dunnet test (p<0.05). SISVAR and SigmaPlot 12 were used for all statistical analysis.

#### **3 RESULTS AND DISCUSSION**

For hybrids KWS9110 (Exp. 1) and 2B587 (Exp. 2) haloxyfop-methyl produced more corn injury than clethodim when herbicides were applied close to crop sowing (2 and 0 DAS). For the hybrid KWS9110, the

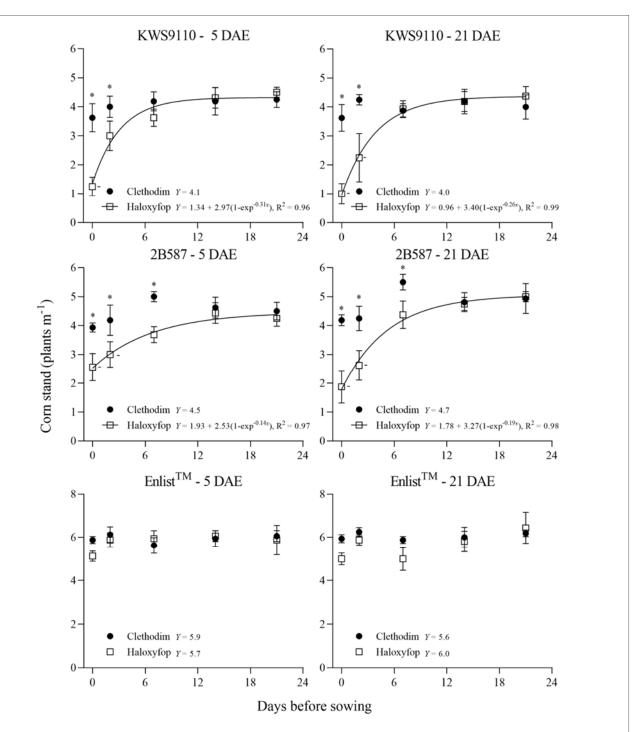
application at the sowing date caused from 25 to 30% crop injury for clethodim and were >75% for haloxyfop-methyl (Figure 2). For the hybrid 2B587, crop injuries caused by clethodim at 0 DAS ranged from 10 to 20% and by haloxyfop-methyl were >60%. As the herbicides were applied with larger intervals between spraying and crop sowing, less crop injury was observed, and <30% of crop injury was found when herbicides were applied with periods of seven or more days before corn sowing for both hybrids. As expected, for the Enlist corn (Exp. 3) no injury symptoms were found after application of haloxyfop-methyl. As expected, very little (<9%) injury was found for clethodim for this hybrid (Figure 2).

For Exp. 1 (hybrid KWS9110), reduced crop stand was observed for those treatments receiving haloxyfop-methyl application (Figure 3). The use of haloxyfop-methyl resulted in significantly lower



\* Significant differences between herbicides (p≤0.05). Dashed lines represents the LSD: least significative difference in relation to untreated check by Dunnet test (p≤0.05). DAE: days after emergence. Bars represent Standard error.

**Figure 2** - Corn injury after clethodim (192 g i.a. ha<sup>-1</sup>) and haloxyfop-methyl (120 g i.a. ha<sup>-1</sup>) application prior to crop sowing. Maringá, 2018.



\* Significant differences between herbicides (p<0.05). - Significant different of untreated check by Dunnet test (p<0.05). DAE: Days after emergence. Bars represent Standard error.

**Figure 3** - Corn stand for hybrids KWS9110, 2B587 and Enlist<sup>™</sup> as a function of time interval between application of clethodim (192 g a.i. ha<sup>-1</sup>) and haloxyfop-methyl (120 g a.i. ha<sup>-1</sup>) application and crop sowing. Maringá, 2018.

corn population as compared to clethodim when herbicide applications were performed at 0 and 2 DBS, both at 5 DAE and 21 DAE. When herbicides were sprayed at 7, 14 or 21 DBS, the differences between herbicides were not significant.

Crop stand was not affected by the time interval between clethodim application and sowing of hybrid KWS9110. In contrast, haloxyfop-methyl decreased significantly corn stand as a function of dates of application (Figure 3).

Haloxyfop-methyl also caused the greatest losses in crop stand as compared to clethodim for the hybrid 2B587 (Exp. 2). When herbicide application was carried out at 0 DBS, the decrease in crop stand may reach 1.38 (5 DAE) and 2.31 (21 DAE) plants m<sup>-1</sup>. At 2 DAS, the difference between the two herbicides was of 1.19 (5 DAE) and 1.63 (5 DAE) plants m<sup>-1</sup>. Still, for herbicides applied at 7 DBS, lower corn density was found for haloxyfop-methyl as compared to clethodim (1.31 at 5 DAE and 1.13 plants  $m^{-1}$  at 21 DAE) (Figure 3).

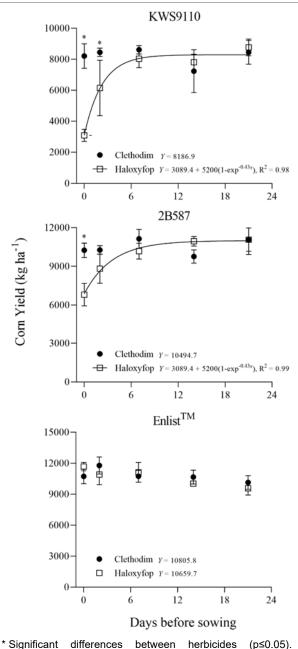
Similar to Exp. 1, the application date of clethodim prior to sowing hybrid 2B587 did not influence corn stand. Regarding haloxyfop-methyl, an exponential model was also fitted between application dates and crop stand (Figure 3). In Exp. 3 (Enlist corn), there were no differences between herbicides clethodim and haloxyfop-methyl as well as for dates of application in relation to corn stand (Figure 3).

The only differences found for plant height were at 21 DAE in Exp. 2 (hybrid 2B587). Application of clethodim resulted in corn plants 2.9 cm shorter than haloxyfop-methyl, when the herbicides were applied at 0 DBS. However, there was no influence of application date on plant height for both herbicides, in all three experiments (data not shown). Plant height means at 21 DAE were 11.7, 13.4 and 14.8 cm for KWS9110, 2B587, and Enlist hybrids, respectively.

Haloxyfop-methyl applied at 0 DBS caused yield losses of 5121.1 kg ha<sup>-1</sup> compared to untreated treatment and of 2300.6 kg ha<sup>-1</sup> when both herbicides were applied at 2 DBS for hybrid KWS9110 (Figure 4). Based on the estimate of maximum yield provided by the model and considering a maximum acceptable yield loss of 2%, the period between haloxyfop-methyl application and hybrid KWS9110 was of eight days (Figure 4).

For the hybrid 2B587, significative differences between herbicides were only found at 0 DBS, when haloxyfop-methyl decreased crop yield by 3443.5 kg ha<sup>-1</sup> compared to clethodim (Figure 4). For this hybrid, and accepting a maximum yield loss of 2%, the interval between haloxyfop-methyl spraying and crop sowing was estimated at 11 days (Figure 4). In Exp. 3, (Enlist hybrid), no yield reduction was found with the application of both herbicides, despite when they were applied.

Haloxyfop-methyl caused more damage than clethodim in hybrids without tolerance to this herbicide. When this herbicide was applied close to the sowing date, increased corn visual injuries, significant crop stand reductions and, therefore, significant yield reductions were observed (Figures 2, 3 and 4). In similar research, where other ACCase inhibitors were applied, yield losses around 500 kg ha<sup>-1</sup> were found with application of fluazifop-p-buthyl (150 g a.i. ha<sup>-1</sup>) and quizalofop-p-ethyl (72 g a.i. ha<sup>-1</sup>) between seven



\* Significant differences between herbicides (p≤0.05). - Significant different of untreated check by Dunnet test (p≤0.05). Bars represent Standard error.

**Figure 4** - Corn grain yield of hybrids KWS9110, 2B587 and Enlist<sup>IM</sup> as a function of time interval between clethodim (192 g a.i. ha<sup>-1</sup>) and haloxyfop-methyl (120 g a.i. ha<sup>-1</sup>) application and crop sowing. Maringá, 2018.

and one day before sowing two different corn hybrids (Mahoney et al., 2016). So far, no previous studies have reported residual effects of haloxyfop-methyl for subsequent corn sowing.

For clethodim applied at 96 g a.i. ha<sup>-1</sup>, Spader et al. (2012) did not observe any crop injury when corn was sown seven and two days after application. At higher doses (136 g a.i. ha<sup>-1</sup>), despite of low levels (<5%) of corn injuries, no effect on grain yield was found when clethodim was applied at seven DBS (Lancaster et al., 2018). In the present work, regardless the

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injury symptoms after clethodim application close to sowing, no significant effect was found in corn stand, height or yield, suggesting that its eventual carryover was not enough to impact corn. There is evidence that clethodim is completely degraded in soil in less than one day (You et al., 2014). Remaining residues in top soil are still exposed to photodegradation (Sandín-España et al., 2016). A typical soil degradation half-life (DT50) for haloxyfop-methyl is 9 days. In practice, the ester form is rapidly cleaved in the environment liberating the free acid haloxyfop-P, which has a typical DT50 somewhat longer from 9 to 21 days (FAO, 2018). The ester form is estimated to be non-mobile in soil, but the acid form is moderately mobile (Vencil, 2002). The combined effect of haloxyfop acid and ester forms may represent enough potential to provide some level of toxicity to corn. Those properties could help to explain the increased damage caused by haloxyfop-methyl in relation to clethodim applied prior to corn sowing.

Soil chemical properties such as pH, organic matter content, cationic exchange capacity (CEC) and texture influence sorption and degradation of herbicides applied to soil (Cornelius and Bradley, 2017). The carryover effect of some herbicides also depends on climatic events like rainfall after application. Usually, rainfall depths between 13 and 19 mm aid herbicide movement into superficial soil layers, providing improved contact with seeds (Riar et al., 2012). For ACCase inhibitors (quizalofop, clethodim, fluazifop, sethoxydim and fenoxaprop), more injuries were found in corn sowed right after application when crop was irrigated (13 mm) after applications, as compared to treatments with no irrigation (Lancaster et al., 2018). In the present work, a significant volume of rainfall was accumulated from the first application at 21 DBS to the last application at 0 DBS (124 mm) (Figure 1).

For the hybrids KWS9110 and 2B587, the application of haloxyfop-methyl should be recommended with the minimum interval of 8 and 11 days before sowing, respectively. Even though results found in the present work may be influenced by local conditions, such as soil chemical and physical aspects, rainfall, soil cover, herbicide doses or hybrid, they suggest the need of taking into account the carryover of this herbicide for corn hybrids without the AAD-1 trait.

The interval between soybean harvest and corn sowing in intensive grain production areas of Brazil is very short or none. In areas where clumps of sourgrass are present at the soybean harvest time and where a high dose of ACCase herbicides are needed, hybrids with tolerance to FOP herbicides such as those carrying the AAD-1 trait may represent an important advantage to farmers, once they may be sowed immediately after herbicide application.

#### **4** CONCLUSIONS

Under the conditions that these experiments were carried out, the estimation of safe period of time between application of haloxyfop-methyl (125 g a.i. ha-1) and corn sowing should be at least of eight days for the hybrid KWS9110 and of 11 days for the hybrid 2B587. There was no influence of time interval between application of clethodim (192 g a.i. ha<sup>-1</sup>) and sowing on corn yield of three hybrids in this study. Haloxyfop-methyl caused more visual damage, increased stand reduction and decreased grain yield than clethodim in hybrids KWS9110 and 2B587, especially when applied next to corn sowing. Enlist<sup>™</sup> corn hybrid demonstrated tolerance to haloxyfop-methyl, indicating that it is safe to sow this hybrid right after treatment.

#### **5** CONTRIBUTIONS

RRM: conceptualization, data acquisition, field research conduction, writing draft and review. LHMF: conceptualization, field research conduction. FRL: funding support, conceptualization, project coordination. LHSZ: conceptualization, funding support, writing review. RSOJr: writing review, funding support, conceptualization.

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