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The effect of spray solution storage time on nicosulfuron efficacy applied in *Urochloa brizantha* cv. Marandu

Efecto del tiempo de almacenamiento de la solución de pulverización sobre la eficacia del nicosulfuron aplicado en *Urochloa brizantha* cv. Marandu

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Resumo

O nicosulfuron é o herbicida comumente utilizado para o controle de plantas daninhas anuais e espécies de folhas largas na produção de milho. No entanto, pouco se sabe sobre o período de tempo em que o nicosulfuron pode permanecer no tanque de pulverização antes da aplicação no campo sem afetar a eficácia. O objetivo deste estudo foi avaliar o efeito de diferentes tempos de armazenamento da solução de pulverização do nicosulfuron na fitotoxicidade, produção e fisiologia do capim-marandu [*Urochloa brizantha* (Hochst. Ex A. Rich.) R. D. Webster cv. Marandu]. O experimento foi analisado em esquema fatorial 6×5 , com delineamento inteiramente casualizado. Os tratamentos foram seis tempos de armazenamento da solução de nicosulfuron (0, 6, 12, 24, 48 e 72 h) aplicada no capim-marandu e cinco períodos de avaliação (0, 7, 14, 21 e 60 dias após a aplicação). A fitotoxicidade pelo nicosulfuron no capim-marandu teve padrão parabólico em função da interação entre o tempo de armazenamento e o período de avaliação. A taxa fotossintética diminuiu em função dos dias após a aplicação do nicosulfuron e teve um discreto aumento em função do tempo de armazenamento da solução de pulverização. A solução de nicosulfuron acidifica com o tempo e os efeitos na fitotoxicidade diminuem em 30% após 72 h.

Palavras-chave: Capim-marandu; Pulverização tardia; Degradação de herbicidas; Taxa fotossintética; Fitotoxicidade.

Abstract

Nicosulfuron is the commonly used herbicide for the control of annual weeds and broadleaf weed species in maize production. However, little is known about the length of time nicosulfuron can remain in the spray tank prior to application in the field without impacting efficacy. The objective of this study was to evaluate the effect of different storage times of the nicosulfuron spray solution on phytotoxicity, production and physiology of Marandu palisadegrass [*Urochloa brizantha* (Hochst. ex A. Rich.) R. D. Webster cv. Marandu]. The experiment was analyzed in a 6×5 factorial arrangement with a complete randomized design. The treatments were six storage times of the nicosulfuron spray solution (0, 6, 12, 24, 48 and 72 h) applied in the Marandu palisadegrass, and five evaluation periods (0, 7, 14, 21 and 60 days after application). Phytotoxicity by the nicosulfuron in the Marandu palisadegrass had parabolic pattern as a function of the interaction between storage time and evaluation period. The photosynthetic rate decreased as a function of the spray solution storage time. Nicosulfuron solution acidifies with time and the effects on phytotoxicity decreased by 30% after 72 h.

Keywords: Marandu palisadegrass; Delayed spraying; Herbicide degradation; Photosynthetic rate; Phytotoxicity.

Resumen

Nicosulfuron es el herbicida de uso común para el control de malezas anuales y especies de malezas de hoja ancha en la producción de maíz. Sin embargo, no se sabe mucho respecto al tiempo que el nicosulfuron puede permanecer en el tanque de pulverización antes de la aplicación en el campo sin afectar la eficacia. El objetivo de este estudio fue evaluar el efecto de los diferentes tiempos de almacenamiento de la solución de pulverización de nicosulfuron sobre la fitotoxicidad, la producción y la fisiología de la hierba Marandu [Urochloa brizantha (Hochst. Ex A. Rich.) R. D. Webster cv. Marandu]. El experimento se analizó en una disposición factorial de 6×5 con un diseño aleatorio completo. Los tratamientos fueron seis tiempos de almacenamiento de la solución de pulverización de nicosulfuron (0, 6, 12, 24, 48 y 72 h) aplicada en la hierba Marandu, y cinco períodos de evaluación (0, 7, 14, 21 y 60 días después de la aplicación). La fitotoxicidad por el nicosulfuron en la hierba Marandu tuvo un patrón parabólico en función de la interacción entre el tiempo de almacenamiento y el período de evaluación. La tasa fotosintética disminuyó en función de los días posteriores a la aplicación de nicosulfuron y mostró un aumento discreto en función del tiempo de almacenamiento de la solución de pulverización. La solución de nicosulfuron se acidifica con el tiempo y los efectos sobre la fitotoxicidad disminuyeron en un 30% después de 72 h.

Palabras clave: Hierba Marandu; Pulverización retrasada; Degradación de herbicidas; Tasa fotosintética; Fitotoxicidad.

1. Introduction

Worldwide, maintaining the productivity and profitability of crops on a commercial scale is very dependent on the use of pesticides (Sharma et al., 2018). However, whether in terrestrial or aerial spraying, success in phytosanitary treatment depends not only on products quality, but also on the technology developed for their application. The need for more economical herbicide applications and environmentally safe is a major concern in agricultural industry (Butts et al., 2019).

Several problems in the field can delay the application of the herbicides decreasing its effectiveness, because some herbicides cannot remain in spray solutions for extended periods of time (Eure et al., 2013). In practice, equipment failure, high wind speed and excessive rain

may lead to the delay of the application (Brabham et al., 2019).

Nicosulfuron is a sulfonylurea herbicide with a pKa of 4.3 and its chemical degradation can occur mainly through oxidation, reduction and hydrolysis reactions (Green & Hale, 2005). According to Benzi et al. (2011), the hydrolysis has been the main degradation factor of the sulfonylurea herbicides due to the substitution reactions of radicals by hydroxyl from the water molecules.

Nicosulfuron is the commonly used herbicide for the control of annual weeds, broadleaf weed species and perennial grasses in intercropped systems, mainly of the *Urochloa* P. Beauv. [syn. *Brachiaria* (Trin.) Griseb.] genus in consortium with corn (Santos et al., 2020; Cholette et al., 2019). However, information in the peer-reviewed literature is not evident relative to the impact of the length of time that nicosulfuron can remain in the spray tank prior to application (Stewart et al., 2009; Eure et al., 2013). We hypothesized that storing the nicosulfuron spray solution would result in reduced effect on plants. Therefore, in this work were evaluated the effects of different storage times of the nicosulfuron spray solution on the physiology and production of palisadegrass {*Urochloa brizantha* (Hochst. ex A. Rich.) R. D. Webster cv Marandu [syn. *Brachiaria brizantha* (A. Rich.) Stapf]}.

2. Methodology

2.1 General description of the experiment

The plants were grown in polyethylene pots of 8 litres (22-cm diameter and 23-cm length) capacity in a greenhouse at JK Campus of the Federal University of the Jequitinhonha and Mucuri Valleys in Diamantina, Minas Gerais, Brazil (18°14'58"S and 43°36'01"W and 1183 m above sea level). The average temperature inside the greenhouse was 22°C, with a maximum of 39 and a minimum of 9°C in the period between August 2016 and July 2017. Two tests were performed, the first batch sown on August 2016 and the second sown on February 2017.

Experiment was analyzed in a 6×5 factorial arrangement with a complete randomized design and seven replicates for each assay. The treatments were six storage times of the nicosulfuron spray solution (0, 6, 12, 24, 48 and 72 h) applied in the palisadegrass, and five evaluation periods (0, 7, 14, 21 and 60 days after nicosulfuron application).

Soil used in the pots was classified as dystrophic red-yellow latosol with clay-sandy texture (sand = 35%; clay = 53%; silt = 12%). Soil chemical analysis resulted in the following

data: pH (H₂O) = 5.12; P = 1.02 mg dm⁻³; K = 25.3 mg dm⁻³; Ca²⁺ = 0.4 cmol_c dm⁻³; Mg²⁺ = 0.2 cmol_c dm⁻³; Al³⁺ = 0.76 cmol_c dm⁻³; H+Al = 5.20 cmol_c dm⁻³; BS = 0.66 cmol_c dm⁻³; OM = 0.70 dag kg⁻¹. One hundred and twenty grams of dolomitic limestone, 500 g of single superphosphate, 30 g of ammonium sulphate and 60 g of potassium chloride were applied in 100 kg of soil as recommendation in pasture (Ribeiro et al., 1999).

Palisadegrass was sown at a depth of 1 cm, leaving two seedlings per pots. The herbicide was applied at 120 days after sowing, at which time the plants of palisadegrass was with average of 63 cm high and 32 tillers. The commercial product Sanson® 40 (ISK Biosciences, SP, Brazil) was used at the dose recommended by the manufacturer for control of *Urochloa* genus in corn (60 g a.i. ha⁻¹ of nicosulfuron). Herbicide was applied using a CO₂-pressurized knapsack sprayer calibrated to deliver 200 L ha⁻¹ aqueous solution at 210 kPa using flat fan 110-02 XR nozzles. The pots were irrigated daily, maintaining soil moisture at 80% of field capacity. Covered fertilizations were done, every 14 days, with 2.5 g of 20-5-20 (N-P₂O₅-K₂O) formulation per pot.

Nicosulfuron spray solution was prepared in translucent polyethylene containers wrapped with aluminum foil and stored indoors at room temperature; the treatments were prepared in decreasing order according to storage times and all solutions were applied at the same time. The chemical analysis of the water used in the spray solutions resulted in the following data: pH = 6.71; $Ca = 5.45 \text{ mg L}^{-1}$; $Mg = 6.12 \text{ mg L}^{-1}$; $Na = 3.97 \text{ mg L}^{-1}$; $K = 1.21 \text{ mg L}^{-1}$; $Mn = 0.73 \text{ mg L}^{-1}$; $Cl = 12.0 \text{ mg L}^{-1}$.

2.2 Measurements

The pH of the spray solution was measured at the time of herbicide application by using a bench pH meter (mPA 210 MS, Tecnopon®, SP, Brazil).

Phytotoxicity of the Marandu palisadegrass was visually estimated at 0, 7, 14, 21 and 60 days after nicosulfuron application (DAA). Phytotoxicity estimates were made on a scale of 0 to 100%, from no apparent plant injury to complete death.

Plant height (base to apex) was measured using a graduated ruler at 0, 7, 14, 21 and 60 DAA. The aboveground biomass was harvested at 60 DAA in all plants by cutting stems and leaves at the soil surface. Subsequently, number and length of tillers were measured per plant and the material was dried at 60°C until constant weight.

Physiological evaluations were performed on the youngest fully expanded leaf at 0, 7, 14, 21 and 60 DAA. To evaluate the photochemical efficiency of photosystem II (relationship

between initial and maximum fluorescence and electron transport rate), the chlorophyll fluorometer (Junior-Pam Walz, Effeltrich, Germany) was used; this measurement was performed at night, after 30 min of dark adaptation. Photosynthesis was measured with a portable photosynthesis analysis system (LCi-SD ADC BioScientific, Hoddesdon, UK). The light intensity in the chamber was kept constant at a photosynthetic photon flux density of 1400 mmol photons⁻¹ m⁻² s⁻¹. The readings were carried out on three leaves per experimental unit on sunny days between 8 and 11 am.

2.3 Statistical analysis

It was made a quantitative study as stated by Pereira et al. (2018). Data were analysed using the means from two growth periods. The phytotoxicity, growth and physiology data of the Marandu palisadegrass were analysed according to the statistical model:

 $Y_{ij} = \mu + C_i + T_j + C {\times} T_{ij} + e_{ij}, \label{eq:constraint}$

where Y_{ij} is the dependent variable; μ is the overall mean;

 C_i is the fixed effect of storage time (i = 1 to 6);

 T_j is the fixed effect of the evaluation period (j = 1 to 5); C × T_{ij} is the effect of factor interaction; and e_{ij} is the residual error.

When the effect of storage time and evaluation period were significant, the comparisons were made using regression analysis. When the interaction between factors was detected, response surface analysis was made using the software SigmaPlot 12.5 (Systat Software, California, USA).

The dry biomass production data of the Marandu palisadegrass were analyzed according to the statistical model:

 $Y_i = \mu + C_i + e_i,$

Where:

 Y_i is the dependent variable; μ is the overall mean;

 C_i is the fixed effect of storage time (i = 1 to 6); and e_i is the residual error.

When the effect of storage time was significant, comparisons were made using the Tukey-test. All statistical analyses were performed using software SAS 9.1 (Statistical Analysis Software, Inc., Cary, NC) and $\alpha = 0.05$

3. Results

The pH of nicosulfuron spray solution was affected (p<0.001) by storage time. The pH values of the spray solution as a function of storage time is shown in Figure 1.

Figure 1. Pattern of pH reduction as a function of storage times (ST) of the nicosulfuron spray solution. pH of water = 6.98.



Source: Research data.

The longer the nicosulfuron is stored, pH of the solution decreases, and this reduction was 0.27 at time zero and 2.15 after 72 h of storage (pH of the solution = $0.0002 \times ST^2 - 0.0415 \times ST + 6.758$; R² = 0.99).

Phytotoxicity of palisadegrass was affected by storage time (p<0.001), evaluation period (p<0.001) and interaction between these two factors (p<0.001; Figure 2).

Figure 2. Effect of interaction between days after application and storage time of the nicosulfuron spray solution on phytotoxicity of Marandu palisadegrass.



Source: Research data.

Phytotoxicity by the nicosulfuron had parabolic pattern as a function of the interaction between storage time and evaluation period (Phytotoxicity = $2.12 - 0.28 \times ST + 1.48 \times DAA - 0.008 \times DAA^2$; R²-adjusted = 0.97). A decrease in plant injury was observed with increasing storage times. Application of the nicosulfuron spray solution without storage (0 h) resulted in 7.5% of phytotoxicity in the Marandu palisadegrass and after 72 h of solution storage, was 1.5% at 7 DAA, reduction of 80% of the nicosulfuron effectiveness in the phytotoxicity. At 60 DAA, the efficacy of nicosulfuron was reduced by 29.1% between 0 and 72 h storage.

There was significant effect of storage time on basal tiller number (BTN; p=0.024) and aerial tiller number (ATN; p=0.040). There was no significant effect of storage time on dry mass production (DMP; p=0.908), basal tiller length (BTL; p=0.095) and aerial tiller length (ATL; p=0.968; Table1). Basal tiller number increased as a function of spray solution storage time at 60 DAA. Spray solution stored at 0 and 72 h produced 66.4 and 86.2 basal tillers, respectively. In contrast, ATN decreased as a function of storage time and solution stored at 0 and 72 h produced 199.8 and 142.6 aerial tillers, respectively. The basal tiller length (BTL) was longer when the storage time for nicosulfuron was increased. However, ATL was not affected significantly, with an average of 12 cm per tiller. The DMP was not influenced by the storage times and higher DMP was observed for the treatment without nicosulfuron application (Table 1).

Table 1. Structural traits of Marandu palisadegrass applied with nicosulfuron at differentspray solution storage times evaluated at 60 days after application.

Storage time (h)	ATN [*]	BTN	ATL	BTL	DMP
0	199.8ª	66.4 ^b	11.87 ^a	26.6 ^b	332.8 ^a
6	148.2 ^{ab}	66.6 ^b	11.24 ^a	27.2 ^{ab}	339.1ª
12	148.3 ^{ab}	70.0 ^{ab}	11.91ª	27.4 ^{ab}	341.3ª
24	146.3 ^{ab}	71.4 ^{ab}	11.88 ^a	27.4 ^{ab}	341.3 ^a
48	143.5 ^{ab}	71.6 ^{ab}	11.14 ^a	31.8 ^a	348.1 ^a
72	142.6 ^b	86.2ª	11.57ª	32.1ª	350.2ª
CV [†] (%)	23.9	16.2	16.04	15.8	7.78
P-value	< 0.001	< 0.001	0.505	0.016	< 0.001
Control [‡]	42.2	142.0	13.4	36.5	456.8

^{a-b} Tukey's-test was used to separate means (p<0.05). ^{*}ATN = aerial tiller numbe; BTN = basal tiller number; ATL = aerial tiller length; BTL = basal tiller length; DMP = dry biomass production. [†] CV = Coefficient of variation. [‡] Control without application. Means with different letters within a column differ. Source: Research data.

There was significant effect of DAA (p<0.001) on plant height [Figure 3(A)] and did not differ among storage time (p=0.297). There was no significant interaction between DAA and storage time on plant height (p=0.833). A quadratic response pattern was observed on plant height as a function of days after nicosulfuron application (Height = $0.0085 \times DAA^2 0.786 \times DAA + 62.7$; R² = 0.94). Nicosulfuron reduced plant height regardless of the storage time of the solution.

Figure 3. Effect of days after nicosulfuron application on the height (A) and the electron transport rate (ETR) (B) and effects of days after nicosulfuron application (C) and storage time (D) on F_v/F_m ratio of Marandu palisadegrass.



Source: Research data.

There was effect of DAA on electron transport rate [p<0.001; Figure 3(B)]. There were no effects of storage time (p=0.196) and interaction between storage time and days after nicosulfuron application (p=0.811) on electron transport rate. Quadratic response pattern was observed for electron transport rate as a function of DAA (ETR = $0.0089 \times DAA^2 - 0.4888 \times DAA + 22.62$; R² = 0.93).

Storage time (p=0.006) and days after application (p<0.001) affected F_v/F_m ratio. There was no significant interaction between storage time and days after application on F_v/F_m ratio (p=0.244). The effects of storage time ($F_v/F_m = 2 \times 10^{-5} \times ST^2 - 0.0009 \times ST + 0.7803$; $R^2 = 0.51$) and of days after application ($F_v/F_m = 6 \times 10^{-5} \times DAA^2 - 0.0044 \times DAA + 0.8168$; $R^2 = 0.78$) on the F_v/F_m ratio are indicated in Figure 3 (C and D), respectively; quadratic response pattern was observed for the F_v/F_m ratio.

There were effects of spray solution storage time (p<0.001), days after nicosulfuron application (p<0.001), and their interaction (p<0.001) on transpiration rate (E), stomatal conductance (Gs), photosynthetic rate (A) and water use efficiency (WUE) of Marandu palisadegrass. Transpiration rate (Equation = $122.8 / \{1 + [(DAA - 50.0) / 249.6]^2\} \times \{1 + [(ST + 41.46) / 6.52]^2\}; R^2$ -adjusted = 0.97), stomatal conductance (Equation = $0.35 / \{1 + [(DAA + 431.68) / 621.16]^2\} \times \{1 + [(ST + 37.46) / 66.11]^2\}; R^2$ -adjusted = 0.99), photosynthetic rate (Equation = $17.66 / \{1 + [(DAA + 927.86) / 3707.61]^2\} \times \{1 + [(ST + 0.74) / 11.51]^2\}; R^2$ -adjusted = 0.99) and water use efficiency (Equation = $10.84 / \{1 + [(DAA + 1020.16) / 1973.75]^2\} \times \{[(ST+130.25) / 170.08]^2\}; R^2$ -adjusted = 1.00) showed a response pattern fitted as a Lorentzian function (Figures 4 and 5).

Figure 4. Effect of interaction between days after application (DAA) and storage time (ST) of the nicosulfuron spray solution on transpiration rate (A) and stomatal conductance (B) of Marandu palisadegrass.



Source: Research data.

Figure 5. Effect of interaction between days after application (DAA) and storage time (ST) of the nicosulfuron spray solution on photosynthetic rate (A) and water use efficiency (B) of Marandu palisadegrass.



Source: Research data.

The transpiration rate decreased with the days after nicosulfuron application and the storage time had a subtle effect on this variable [Figure 4(A)]. The stomatal conductance decreased as a function of days after nicosulfuron application and spray solution storage time [Figure 4(B)]. The photosynthetic rate decreased as a function of the days after nicosulfuron

application and showed a discrete increase as a function of the spray solution storage time [Figure 5(A)]. As observed for phytotoxicity, although the plants are affected by the herbicide, these results indicate a 46% reduction in the nicosulfuron efficacy after 72 h of storage. The water-use efficiency (WUE) decreased as a function of days after nicosulfuron application and spray solution storage time [Figure 5(B)].

4. Discussion

The most important degradation process of the sulfonylurea herbicides groups is hydrolysis, with direct interference in the final pH of the solution (Benzi et al., 2011). The nicosulfuron molecule dissociates as it remains stored in water and acidifies the solution, this can reduce its effect on management of targeted weeds (Green & Hale, 2005). Hydrolysis of the herbicide nicosulfuron principally involves the breakdown of the urea part of the molecule (Sabadie, 2002).

Nicosulfuron hydrolysis depends on temperature and pH of the solution; the pH controls the hydrolysis because the neutral form of the sulfonylurea binder is considerably more susceptible to hydrolysis than the ionic form (Stewart et al., 2009). In addition, the pH of the spray solution is the dominant factor that affects its biological activity (Holmberg et al., 2003) and nicosulfuron has shown reduced solubility and efficacy at low pH levels (Green & Cahill, 2003). According to Smith and Aubin (1993), the half-life of sulfonylureas chlorsulfuron, chlorimuron-ethyl, metsulfuron methyl and sulfometuron ethyl in water at pH 5 was 1.7, 0.6, 2.1 and 0.4 days, while under similar conditions at pH 7 the half-life were 51, 14, 33 and 6 days, respectively.

In the current study, the efficacy of nicosulfuron was lower as a function of spray solution storage time. The herbicide effectiveness reduction was 30% on visual plant injury after 72 h of storage. However, for the effects on DMP, the reduction in effectiveness was only by 5%. In addition, Stewart et al. (2009) reported no decrease in control of common ragweed (*Ambrosia artemisiifolia* L.), common lambsquarters (*Chenopodium album* L.), redroot pigweed (*Amaranthus retroflexus* L.), and velvetleaf (*Abutilon theophrasti* Medik.) by postemergence application of nicosulfuron plus rimsulfuron when spray solutions were prepared 3 and 7 days prior to application.

Phytotoxicity occurs through the uptake and translocation to the meristematic regions in developing tissues inhibiting the acetolactate synthase enzyme (Eberlein et al., 1997). Nicosulfuron have inhibitory effect on enzyme that acts directly on the apical growth areas of

plants and reduces the apical dominance allowing greater aerial tillering (Rahman & James, 1993). In the present study, this effect in increasing aerial tillering was lower by 30% with 72 h of spray solution storage time, highlighting the reduction of the nicosulfuron effectiveness.

Regarding the physiological characteristics, it is possible to affirm that the spray solution storage time practically did not affect the analyzed variables. Despite the significant interaction between storage time and DAA in the physiological variables, the effect of storage time was very subtle.

All values of F_v/F_m ratio observed for Marandu palisadegrass are close to the optimum range. The maximum quantum yield of photosystem II can range from 0.75 to 0.85 quantum⁻¹ electrons for unstressed leaves (Bolhar-Nordenkampf et al., 1989). This response pattern was expected because ALS-inhibiting herbicides, such as nicosulfuron, does not affect photosystem II pathway.

Solution storage reduces nicosulfuron efficacy on phytotoxicity and structural traits, but not on photosynthesis; probably due to the mechanism of action of nicosulfuron that directly affects plant growth. Acetolactate synthase inhibitor herbicides inhibit the synthesis of branched-chain amino acids valine, leucine and isoleucine, impairing DNA synthesis and cell growth (Duggleby et al., 2008).

There was a decrease in the activity of the electron transport chain after the nicosulfuron application in palisadegrass, regardless of spray solution storage time; however, a possible recovery of the plant to the effects of the herbicide was observed at 60 DAA. In addition, electron transport has a strong linear relationship with the photosynthetic rate (Zhong et al., 2014). The CO₂ uptake by the plants is directly related to their metabolism; injured plants by herbicides have losses in the metabolic efficiency and consequently in the photosynthetic rate (Taiz et al., 2017).

The water-use efficiency associate photosynthesis and transpiration, being the result of the relationship between carbon uptake and water loses by the plant. The opening of the stomata for CO₂ uptake is related to the water loss by the plants; to reduce this loss, the closure of the stomata also restricts the CO₂ uptake. In addition, plants can quickly control the stomatal opening in response to an environment stimulus (Taiz et al., 2017). However, when plant is stressed by the herbicide, there may be a decrease in photosynthetic rates, resulting in lower yields for water use efficiency. Campelo et al. (2015) evaluated the gas exchanges of *Astronium fraxinifolium, Calophyllum brasiliense, Handroanthus serratifolius, Handroanthus impetiginosa, Simarouba amara* and *Swietenia macrophylla*. The authors verified that the reduction of the photosynthetic rate was related to the stomatal closure.

5. Final Considerations

Nicosulfuron solution acidifies with time and the effects on phytotoxicity decreased by 30% after 72 h; however, it did not decrease the effect on the photosynthesis traits.

The decrease in effectiveness with storage of nicosulfuron spray solution should be investigated in grasses intercropped with a field crop, in order to endorse the interference in the crop to be protected.

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