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Determination of the Persistence in Soil of the Herbicide Indaziflam Applied on Sugarcane Crop under Brazilian Conditions

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Abstract: The sugar beet, *Beta vulgaris* L. (cv. Early Wonder), was selected as a plant test for the herbicide indaziflam and used to determine the persistence of this herbicide under field conditions in the sugarcane crop under Brazilian conditions. A randomized block design with four treatments was used: weeded control and Indaziflam 75, 100 and 200 g/ha, arranged in a randomized block design with four repetitions. For the determination of persistence, soil samples were taken at sixteen times: 0, 30, 74, 99, 134, 167, 195, 224, 264, 295, 327, 365, 406, 454, 491 and 522 DAT (Days After Treatments). To determine the persistence, the bioassay methodology was used with sugar beet plant test. The persistence in the soil of Indaziflam, as a function of the treatments was, respectively: 365 DAT, 150 g/ha; 454 DAT, 200 g/ha and 491 DAT, 400 g/ha.

Key words: Bioassay, residue, plant test, environmental contamination.

1. Introduction

In the 1950s, the relationship between crop plants growing together with weeds began to be analysed by scientific and experimental methods.

The adoption of herbicides in Brazil became frequent in the 1970s, during the Green Revolution: adoption of technological innovations in agricultural production chains, involving profound changes in aspects of agronomic innovations, including the intensive use of pesticides, a fact that was accentuated with the introduction of the first transgenic crop—Roundup Ready—soybeans, resistant to the application of glyphosate, since then, accentuating the consumption of pesticides, until the current moment, in which Brazil is among the world's largest consumers of this input [1, 2].

In the year 2016 [3, 4] pesticide sales in Brazil were more than 100 billion dollars corresponding to 641.5 million tons of pesticides, of these, 59.46% corresponded to the class of herbicides directed mainly to soybean, sugarcane and corn crops, respectively [4]. Among these crops, sugarcane—grown on nine million hectare in the 2015/2016 crop year—is probably the one that presents the highest probability of problems of permanence of herbicides in the soil for a longer time than desirable (persistence) due to the fact that it presents a slow initial growth and subject to weed competition, thus requiring herbicides with prolonged residual power (action on weeds), especially when planting is performed at the end of the rainy season—southeast region of Brazil—because the herbicide has to be effective, controlling the first flushes of weed emergence, when the rains return, six months after its application in the crop planting [5].

This is corroborated by studies dating back to the since the end of the 1970s which assessed the interference of weeds in sugarcane crops and determined that the period during which the crop should remain free of weeds is 90 to 120 days after planting, thus proving the need to use herbicides with

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long residual power capable of controlling the weeds during this period [6, 7], after this period, the presence of the herbicide in the soil is no longer desirable because, although present in the soil, its relative low concentration is no longer able to control weeds, but is likely to cause environmental contamination and damage to crops in rotation, thus justifying research that can assess its persistence in soils [8].

Among the methods used to determine the persistence of herbicides in the soil, there is the methodology by chemical extraction, often used to determine the total concentration of the herbicide: available portion for absorption present in the soil solution and its unavailable portion, sorbed to the soil colloids [9]. However, with this method it is not possible to evaluate if the concentration obtained can affect sensitive crops used in rotation [8, 10]. This can be solved by opting for the bioassay method that uses bioindicators: organisms with extreme sensitivity to a given herbicide, with which there is indirect measurement of the herbicide present in the soil, through the sensitization of the plant test, expressed by its bioactivity, determining the period of time (persistence), in which the concentration of herbicide present in the soil solution is likely to be absorbed by sensitive plants (plant tests). The end of this period indicates the minimum threshold for planting sensitive crops in succession or crop rotation system and establishes the period in which the herbicide has the potential to cause environmental contamination [10].

It should be noted that, when bioassays determine the persistence of herbicides in the soil through the methodology with plant tests, they also detect the presence of metabolites of the herbicide with the potential to cause damage to plants, which is not possible with the chemical method, due to its intrinsic characteristics, specificity of detection for a single molecule. This would contribute to explain why, in many cases, the bioassay methodology is more sensitive than the chemical one, being able to detect the presence of the herbicide below the detection limit of this method, since the plant test can be sensitized by the original molecule including its metabolites [5].

It should be stressed that research aimed at determining the persistence of herbicides in the soil is indispensable for characterising their ecotoxicological profile, particularly for new herbicides such as indaziflam.

Indaziflam herbicide ($C_{16}H_{20}FN_5$), IUPAC name: N-[(1R,2S)-2,3-dihydro-2,6-dimethyl-1H-inden-1-yl]-6-[(1RS)-1fluoroethyl]-1,3,5-triazine-2,4-diamine, belongs to the alkylazine chemical group, vapour pressure (25 °C): 5.1×10^{-10} mm Hg; partition coefficient (Log Kow) pH(7): 2.8; Solubility (pH 6.8) 2.8 mg/L and dissociation constant pKa 3.5 (weak acid) [11, 12]. It has a systemic and selective character, its mode of action is the inhibition of CBI (Cellulose Biosynthesis). After the germination of the seeds, it acts in the hypocotyl region of the seedlings causing the loss of the anisotropic growth of the cells, provoking an accentuated expansion of the radial growth, resulting in swelling in this region. It is indicated for broad spectrum control of weeds, monocotyledonous and dicotyledonous, applied as a pre-emergent. In Brazil, it is registered for banana, coffee, cashew, sugarcane, citrus, coconut, dendê, guava, apple, mango and grape crops [11-13].

Currently, research results evaluating the behaviour in soil of indaziflam herbicide are scarce, and indicate that it has long persistence in agricultural soils: half-life > 150 days [14], and 86 days [14], using the methodology of labeled carbon (^{14}C), determined three metabolites of indaziflam: FDAT (Indaziflam-Triazinediamine), ITI (Indaziflam-Triazine-Indanone) and ICA (Indaziflam-Carboxylic Acid), these, in soil under laboratory conditions (no light, 20 °C, aerobic conditions), persisted up to 122 days. [15] evaluated the sorption/desorption of the three metabolites mentioned indicating that they showed different behavior in relation to sorption in soil and that they are less sorbed than Indaziflam (original molecule), showed that they have greater potential for leaching in soil, especially in those with low carbon content and high pH, and also

that the desorption showed hysteresis for all metabolites and in all soils evaluated, observations corroborate those of González-Delgado, A. M., et al. [16], who evaluated the leaching of indaziflam in areas with pecan cultivation and observed fewer phytotoxicity symptoms in soils with higher organic matter content and greater leaching in soils with greater porosity.

Therefore, the research described in this article aims to determine the persistence of the herbicide indaziflam with biological action, applied to the sugarcane crop, contributing to the ecotoxicological understanding of this herbicide, notably under Brazilian conditions.

2. Material and Methods

To achieve the objective proposed here, two trials were conducted: the first, preliminary, to determine specific plant test for the herbicide indaziflam, to be used in the second trial: determining the persistence in soil of the herbicide indaziflam, applied to sugarcane crop.

2.1 Preliminary Trial: Determination of a Specific Plant Test for the Herbicide Indaziflam

Species assessed: soybean (*Glycine max*), crotalaria (*Crotalaria juncea*), sorghum (*Sorghum bicolor*), beans (*Phaseolus vulgaris*), corn (*Zea mays*), sunflower (*Helianthus annuus*) and sugar beet (*Beta vulgaris*).

Individually for each species, bioassays were conducted to evaluate the effect of increasing doses of indaziflam herbicide (treatments), using plastic cups with a capacity of 300 mL without percolation containing 250 g of soil (experimental unit) sown with the seven species individually, in an entirely randomized design with 10 repetitions. These were irrigated daily until 80% of the field capacity, conditioned in a growth chamber (fitotron), Conviron model PVG36, regulated for 20 °C, 70%-80% of relative humidity, photoperiod of 12 h with light intensity, 35,400 lumen/m^2.

After fourteen days, the plants were cut close to the soil and the epigeal fresh masses were obtained for each treatment. The data obtained were submitted to variance analysis and when significant $F(5\%)$ was determined for the logistic-dose-response regression models [17] $F(5\%)$, quantifying the sensitivity of each species evaluated through the RC50 index—concentration that reduces 50% of epigeal fresh mass.

2.2 Second Trial: Determination of Persistence in Soil of the Herbicide Indaziflam Applied to Sugarcane Crops

The experiment under field conditions was carried out in the city of Campinas, state of São Paulo, Brazil, geographical coordinates: 22°54′31.7″ latitude South, 47°01′3.3″ longitude West and altitude, 670 m; climatological classification Köppen-Geiger: *Cfa*: humid subtropical, mean annual temperature: 21.3 °C and annual rainfall: 1,462 mm. Soil: eutrofic red-yellow argissolo, slope 2%, no history of residual herbicide application during the last four years. Chemical characteristics: pH 6.4; organic matter 11 g/dm^3; physical (texture): sand 32%, clay: 39% and silt 11%; cation-exchange capacity CEC: 78.7; *V* (%): 56.8; textural classification: sandy clay.

The sugarcane cultivar CTC 7 at the quantity of 10 t/ha was planted in soil conveniently prepared by one ploughing and two harrowing with fertilization of 500 kg/ha of N P K, 25 25 25, on November 17, 2010.

There were four treatments: a weeding control plus three treatments with the herbicide indaziflam: 75, 100 and 200 g/ha, applied in the form of Alion 500 SC®—concentrated solution containing 500 g of indaziflam per litre—applied as a pre-emergent once only, one day after planting the sugarcane.

The plots were 7.00 m (length) × 5.20 m (width), totaling 36.40 m^2, containing four sugarcane planting lines, spaced by 1.30 m, the experiment was arranged in a randomized block design with four repetitions to determine the persistence of indaziflam the bioassay methodology was used, using as plant test the sugar

beet cv. Early Wonder.

For this purpose, soil samples were taken using a cylindrical steel auger (15 cm diameter by 10 cm high), in a randomized manner in four points per plot, obtaining composite samples for each treatment, in sixteen evaluated periods: 0, 30, 74, 99, 134, 167, 195, 224, 264, 295, 327, 365, 406, 454, 491 and 522 DAT (Days After Treatments). The composite samples were sieved, air-dried and stored in a freezer (-15 °C) until the preparation of the bioassays.

To determine the persistence in soil of the indaziflam herbicide through bioassays, tests corresponding to the respective treatments were performed in each sampled season, sowing the sugar beet cv. Early Wonder in plastic cups (300 mL) without percolation with 250 g of soil, considered as experimental unit, arranged in an entirely randomized design with four repetitions, in a phytotron—Conviron model PVG386—regulated in the following conditions: 20 °C, 75% relative humidity of the air, photoperiod of 12 h and light intensity of 35,400 lumen/m^2; the cups were irrigated daily until 80% of the field capacity.

After 14 days, the test plants were cut close to the ground and the epigeal fresh masses (g) were evaluated, and the data were submitted to variance analysis. When significant (α5%), the test of means t(5%) was performed, evaluating in each season sampled the hypothesis of nullity between the averages of the weeded witness, individually with the averages of the treatments with indaziflam herbicide.

3. Results and Discussion

3.1 Preliminary Trial: Determination of Plant Test for the Herbicide Indaziflam

Among the species evaluated: soybean (*Glycine max*), crotalaria (*Crotalaria juncea*), sorghum (*Sorghum bicolor*), beans (*Phaseolus vulgaris*), corn (*Zea mays*), sunflower (*Helianthus annuus*), sugar beet (*Beta vulgaris*), cultivar Early Wonder, was characterized as the most sensitive to indaziflam herbicide, because it showed the lowest value of RC50. The determination of the dose-response logistic model, characterizing the correlation of the herbicide on sugar beet epigea fresh biomass is described in Fig. 1.

Fig. 1 characterizes the dose-response logistic model including its confidence interval (90%), indicating the correlation between the factors: concentrations of indaziflam (µg/kg), on the epigeal fresh mass of sugar beet (g), vegetating fourteen days under climatic conditions regulated by phytotron. It also established optimum accuracy for the model (R^2 = 0.94), and determined the concentration of the herbicide that reduced by 50% the epigeal fresh mass of the plant test sugar beet (GR50), 0.65 µg/kg, with confidence interval (90%) ranging from 0.55 to 0.79 µg/kg. It is relevant to note that the value of GR50 (0.65 µg/kg) corresponds to 115.3 times the maximum dose indicated for the use of indaziflam (75 µg/kg), distributed in the 0-10 cm soil layer with a density of 1.2 g/cm^3, thus proving the extreme biological sensitivity of sugar beet, cultivar Early Wonder, to the herbicide, justifying its choice as a test plant for the bioassays, with the aim of determining the persistence in the soil of this herbicide.

3.2 Second Test: Determination of the Persistence of Indaziflam in the Soil Applied to Sugarcane Crops

The results of the correlation between treatments on the epigeal fresh mass of the plant test, under phytotron conditions, in the sixteen sampled seasons, are depicted in Fig. 2.

It is observed from Fig. 2 the temporal bioactivity of indaziflam herbicide present in the soil, is expressed in the epigeal fresh mass of the plant test beet during the trial period (523 days). It is indicated that the first expression of development of the plant test only occurred at 99 DAT, for the treatments 75 and 100 g/ha, while for the treatment 200 g/ha, this event occurred 266 days later, at 365 DAT. After these times, a gradual increase in the averages of epigeal fresh masses was observed in all treatments with the indaziflam herbicide until the end of sampling at 523 DAT.

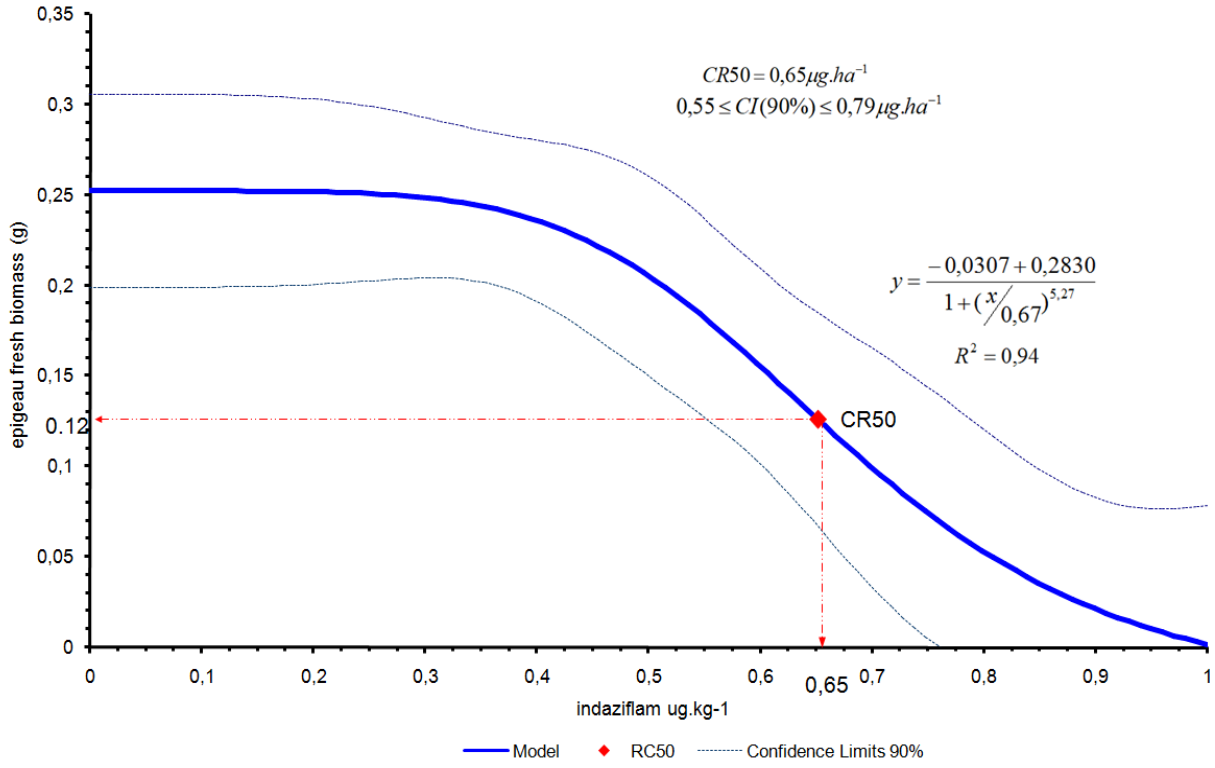

Fig. 1 Sensitivity evaluation of sugar beet (*Beta vulgaris*) cv. Early Wonder submitted to increasing doses of Indaziflam. Mean data from ten repetitions.

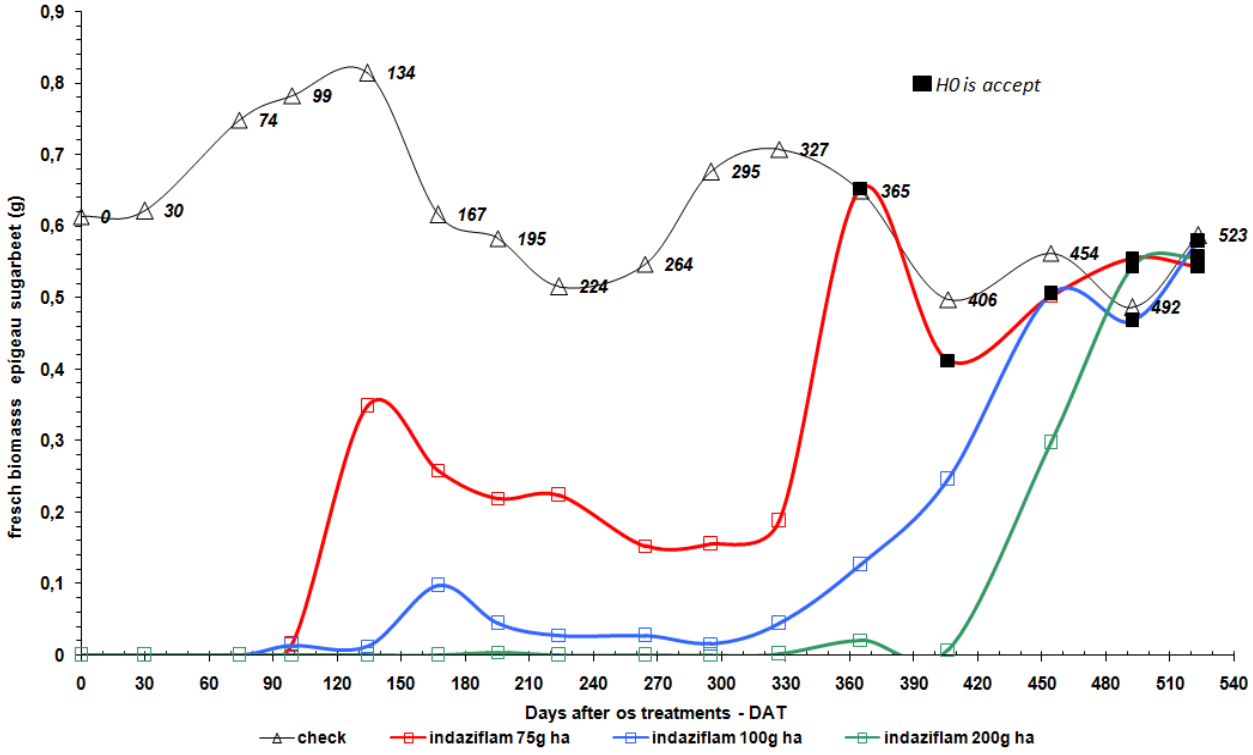

Fig. 2 Temporal variation of epigeal fresh mass of sugar beet as a function of DAT. The symbol ■, represents non-significant difference ($p > 0.05$) in relation to the control.

In this progression of the averages of the treatments with indaziflam herbicide during the trial period, described in Fig. 2, the results of the comparison between the tests of averages are observed, evaluating the null hypothesis (*H0*) during the sixteen seasons evaluated, indicating by symbols its rejection (□), and its acceptance (■), this occurred initially for the treatments: 75, 100 and 200 g/ha, respectively at 365, 454 and 492 DAT, lasting until the end of the evaluations in 523 DAT.

The acceptance of the null hypothesis (■) indicates the inability of the concentration of indaziflam herbicide present in the soil to biologically affect the plant test, thus characterizing the end of persistence of indaziflam herbicide at 365, 454 and 492 DAT, for the respective treatments, 75, 100 and 200 g/ha. These results are relevant because they help in the construction of the ecotoxicological profile of this herbicide, in this case, indicating that it presents long persistence in the soil, susceptible to environmental contamination and with the capacity to affect sensitive crops under tropical conditions.

Among the various factors that influence the dissipation of herbicides in the soil the climatic conditions are relevant. Fig. 3 indicates air temperature, abundance and frequency of rainfall during the trial period.

Fig. 3 shows that at the sampling time the climatic conditions: frequency, abundance and total rainfall, maximum and minimum temperatures were characterized as normal for the time and place of the trial—southeastern Brazil. Through these data it was possible to determine the hydric balance, thus indicating the periods of deficit and excess of water in the soil, in Fig. 4 [18].

Fig. 4 highlights the water balance during the trial period, determined by potential evapotranspiration—process of transferring water from the soil to the atmosphere—indicating the periods in which the soil was in a state of water deficit or excess. This particularity,

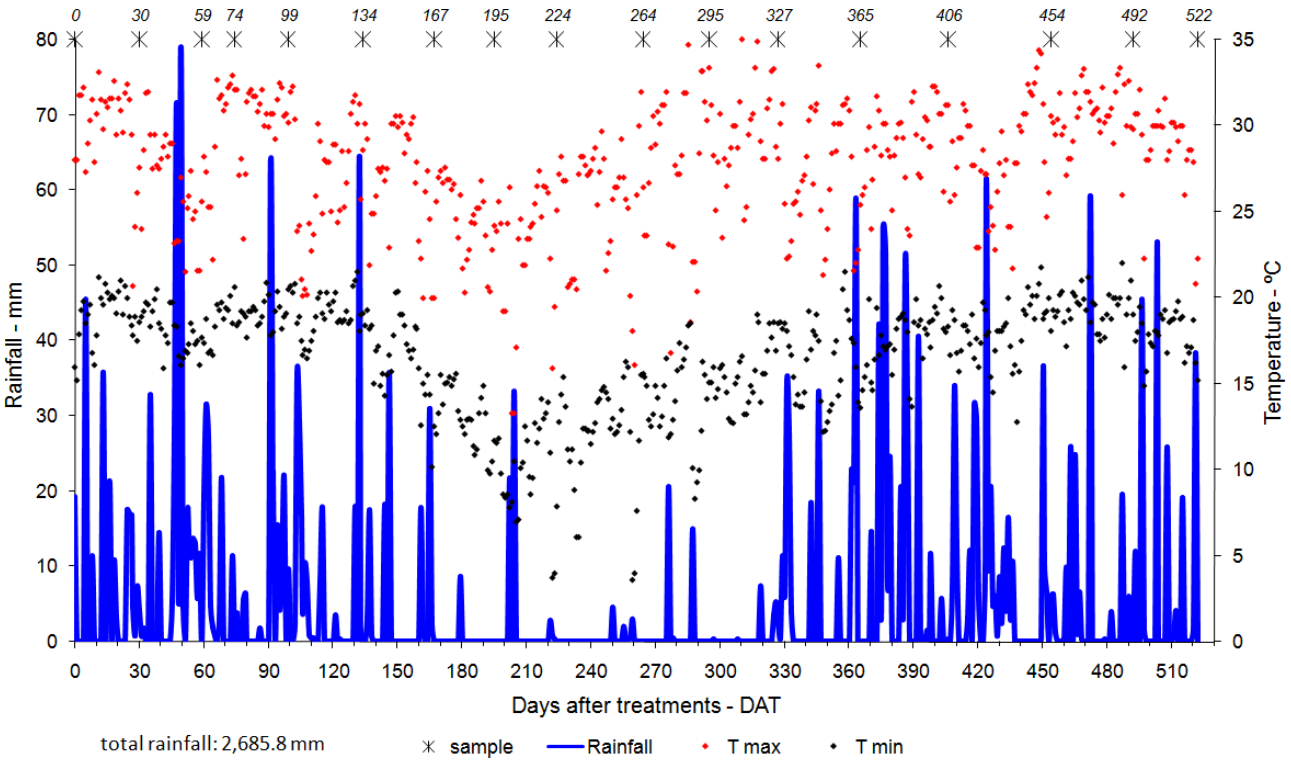

Fig. 3 Climatic conditions: rainfall, maximum and minimum temperature and seasons sampled, during the trial period: 18/11/2010 until 23/04/12.

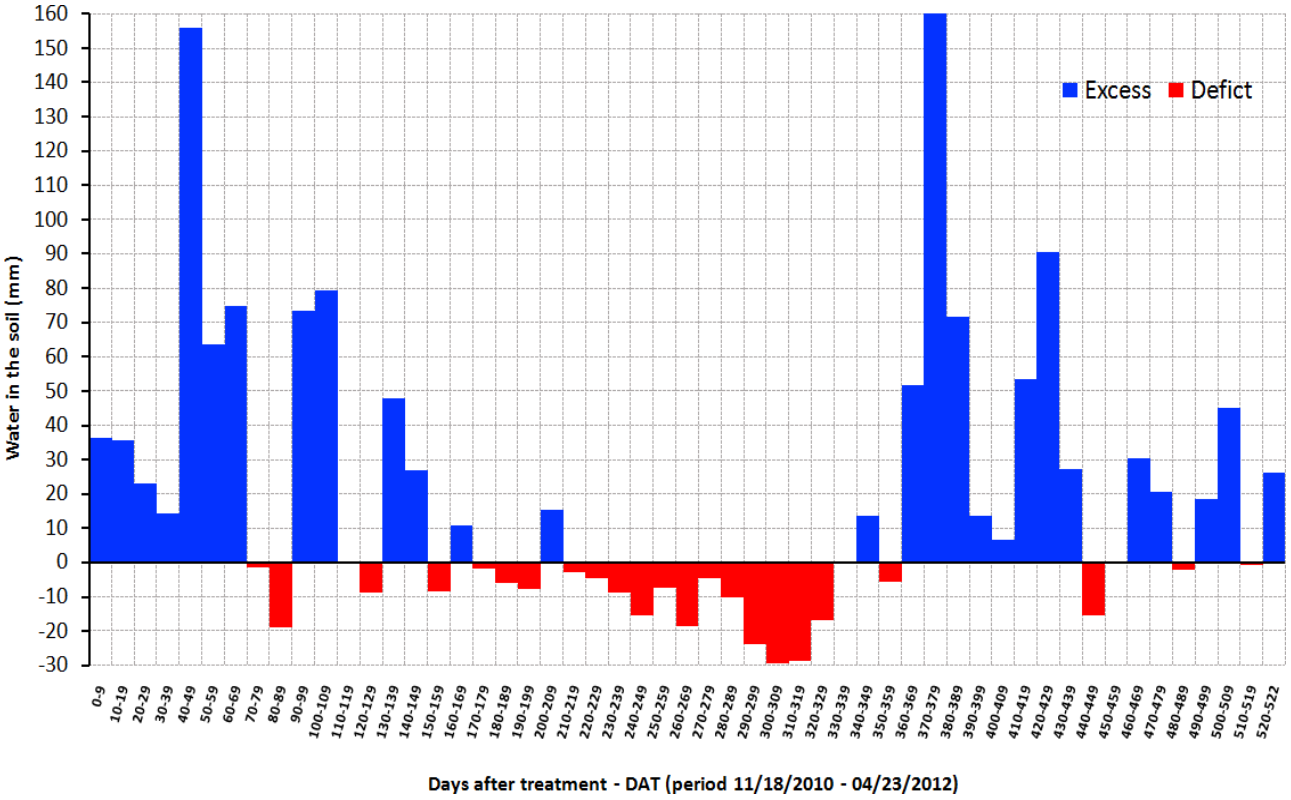

Fig. 4 Water balance every ten days classifying the soil water condition during the test period.

correlated to the soil attributes: pH index, colloid contents (clay and organic matter) and herbicide: molecular structure, ionization and water solubility, lipophilicity, polarization and volatilization are fundamental variables to understand the behavior of herbicides in soils, notably the dynamics of their sorption [19-22].

Being a weak acid, the ionisation of indaziflam is governed by the relationship between its pKa 3.5 and the pH of the soil: 6.4; in this case, it is determined[1] that 99.8% of its molecules are in the anion form, with a tendency to remain in the soil solution, rather than being sorbed to the soil colloids, and thus subject to dissipation processes, including leaching.

Fig. 4 shows that in the initial period of the trial (0 to 169 DAT), the soil was predominantly with excess water, a condition that favoured the desorption of the herbicide molecules from the colloids into the soil solution, corroborating so that the majority of its molecules were in the anion form, thus favouring the dissipative processes and the decrease of its concentration in the soil. This tendency is confirmed by the first manifestation of the development of the plant test beet during this period, 99 DAT, for the treatments 75 and 100 g/ha.

After this period, in the same Fig. 4, it was verified an interval of water deficit initiated at 170 up to 339 DAT, thus favouring the sorption of the indaziflam herbicide, including its ionized form, to the soil colloids, and thus, less accessible for the dissipative processes; This fact lasted until the return of the new cycle of rains at 340 DAT, period in which the soil was in a new cycle with conditions of excess water and thus, favored the desorption and consequently the dissipation of the herbicide, thus, collaborating so that at 365 DAT there was a significant reduction in its concentration in

[1] $\%ionization = \frac{100}{1 - anti\log(pKa - pH)}$.

the soil, and the first expression of the development of the plant test in the treatment of higher concentration: 200 g/ha.

Fig. 4 also shows that it took two periods of excess soil water: 0 to 169 DAT and 340 to 499 DAT, interspersed with one of water deficits: 150-339 DAT for the null hypotheses for the three treatments to be accepted at 365, 454 and 492 DAT; for the respective treatments: 75; 100 and 200 g/ha, characterizing these epochs as the end of persistence in soil of the indaziflam herbicide in the respective treatments applied to sugarcane crop under tropical conditions at the beginning of the rainy season in the southeast region of Brazil.

The persistence results described here are corroborated by the authors [11, 14-16] confirming that indaziflam exhibits long persistence in soil.

In another study, not published here, it was shown that climatic conditions influence the persistence of indaziflam herbicide, because when it was applied under the same soil and climate conditions, but at the end of the rainy season; persistence was significantly greater, compared with when it was applied at the beginning of the rainy season, a situation described in this communication.

4. Conclusion

Beetroot is suitable as a plant test for bioassays to determine the persistence in soil of the herbicide indaziflam.

The herbicide indaziflam when applied to sugarcane crop in tropical conditions shows long persistence with bioactivity in the soil.

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