Australian Journal of Crop Science AJCS

AJCS 17(8):645-652 (2023) ISSN:1835-2707
doi: 10.21475/ajcs.23.17.08.p3920

# Potential of green manure in the phytoremediation of the herbicide indaziflam after different times of application

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**Abstract**

The identification of species that promote phytoremediation of herbicides is of great relevance to reduce the impact of these products on the environment. This study aims to evaluate the efficiency of plant species in the phytoremediation of the indaziflam herbicide. The phytoremediation potential for indaziflam of 15 species of green manure was evaluated at different sowing times of the *Phaseolus vulgaris* (bioindicator), after the herbicide application. *P. vulgaris* was sown after the removal of the aerial part of the selected green manure. The experiment was conducted in a greenhouse, and each green manure species was evaluated separately in a completely randomized design, with four replications. The treatments were arranged in a 3 x 5 factorial layout, consisting of three doses of indaziflam (0, 75 and 100g a.i. ha$^{-1}$) and five sowing times (5, 30, 60, 90 and 120 days after the herbicide application). According to the phytotoxicity and biometric evaluations of green manure, the potential species for the phytoremediation of the indaziflam were selected. The results showed that, apart from the species *Lupinus albus*, *Canavalis ensiformis* and *Mucuna cinerea*, the other green manure evaluated were highly susceptible to the herbicide indaziflam. However, although these potential species presented less control by indaziflam, none of them were effective in their soil phytoremediation. The bioindicator showed high phytotoxicity and reduction of dry mass due to the herbicide, regardless of the green manure sowing season on both doses of indaziflam. As a phytoremediator, none of the green manure species were effective for indaziflam even after 120 days of the herbicide application.

**Keywords**: cover plants; preemergent; residual; selectivity; susceptibility.
**Abbreviations:** a.i._active ingredient; Dry M._dry matter; DAA_days after application; DAE_days after emergence.

## Introduction

The herbicides used in preemergence, also called residual herbicides, prevent, or reduce the emergence of weeds from the seed bank. This category of herbicides has shown an increase in its use and is important to ensure the productivity of commercial crops, as they prevent competition between weeds and crops during the initial stages of development (Procópio et al., 2008). However, it is necessary to note the contamination of environments due to the permanence of these products in the soil. Noticeable direct effects include symptoms of phytotoxicity and reduced productivity of sensitive crops that are sow in succession/rotation caused by long lasting residual herbicides (Mancuso et al., 2011).
An important characteristic of the herbicide indaziflam is its high residual period in the soil, of more than 150 days, persisting for longer when compared to several other herbicides applied in preemergence (Kaapro and Hall, 2012). Although it allows greater flexibility regarding the time of application, it can cause environmental problems due to the extended period it is present in the environment, such as carryover and water contamination by leaching.
Studies show high persistence of this herbicide in the soil, and it was verified that even after 100 days of the indaziflam application (100g a.i. ha$^{-1}$), regardless of sandy or clayey soil, it resulted in the death of the bioindicator (cucumber) (Savaris et al., 2019).
Techniques such as bioremediation, phytoremediation, chemical oxidation, surfactant-based extraction, electrokinetic remediation, and thermal desorption are used for decontamination of polluting substances in the environment. Due to the excessive cost, the need for specific equipment and possible effects on the biological and chemical quality of the soil some of these methods are rarely used (Lim et al., 2016).
Interest in phytoremediation has increased in recent years. This technique can minimize the damage caused by carryover to subsequent crops, reduce the impact of herbicide residues on soil and groundwater, and reduce the release time of the area for planting different plant species weak to persistent compounds (Procópio et al., 2008).
Phytoremediation consists in the use of plants, their associated microbiota and soil softeners such as correctives,

fertilizers, organic matter, etc. in addition to agronomic practices that, if applied alongside, remove, immobilize, or make contaminants innocuous to the ecosystem (Accioly and Siqueira, 2000). The use of this technique has shown promising results for heavy metals, metalloids, petroleum hydrocarbons, pesticides, explosives, chlorinated solvents, and toxic industrial byproducts. Research has also spread the use of plants in herbicides phytoremediation (Vasconcelo et al. 2020; Madalão et al., 2013; Monquero et al., 2013; Santos et al., 2006).

The use of phytoremediation is based on the natural or developed, selectivity of some species, to certain types of substances or mechanisms of action, commonly occurring in species tolerant to certain herbicides. This selectivity is due to the fact that organic compounds can be moved to other tissues of the plant and then volatilized; suffer from partial or complete degradation or can be transformed into less toxic substances, combined and/or bonded to plant tissues (compartmentalization) (Accioly and Siqueira, 2000; Scramin et al., 2001; Santos and Ribeiro, 2019).

According to Scramin et al. (2001) some weeds may have the potential for phytoremediation of herbicides in soils, however, it is recommended to select easily controllable species. Thus, most studies are focused on species used as green manure (Pires et al., 2005; Procópio et al., 2005; Monquero et al., 2013). Since there is no need to remove the plant after the remediation process, the biomass produced by the green manure during the phytoremediation process can be used to benefit the production system. Therefore, in addition to phytoremediation, these species can also benefit physical, chemical and biological characteristics of the soil.

Green manure such as *Canavalis ensiformis*, *Raphanus sativus*, *Crotalaria spectabilis*, *Lupinus albus* and *Pennisetum glaucum* are described as candidates in phytoremediation of organic pollutants because they have rapid growth, high biomass production and the ability to improve soil conditions for following crops (Santos, 2018).

The objective of this article was to evaluate 15 potential species of green manure in the phytoremediation of the herbicide indaziflam at different doses and sowing times after the application.

**Results and Discussion**

***Selection of plants with potential for phytoremediation of soils contaminated by the herbicide indaziflam***

The green manure species *Lolium multiflorum* did not germinate at any period of sowing, therefore there was a 100% phytotoxicity in all treatments, thus, the data referring to this species were not presented.

The results obtained regarding the phytotoxicity of green manure, at doses 0,75 and 100g a.i. $ha^{-1}$ of indaziflam, according to the predetermined sowing times of the species, are presented in Table 1S.

For most species of green manure (*Lupinus albus*, *Raphanus sativus*, *Avena strigosa*, *Secale cereale*, *Crotalaria breviflora*, *Crotalaria spectabilis*, *Crotalaria juncea*, *Sorghum bicolor*) there was no statistically significant difference between the sowing periods, regardless of the dose applied. Minimum differences were also verified for the species *Canavalis ensiformis*, *Vicia sativa*, *Helianthus annuus*, *Fagopyrum esculentum* and *Pennisetum glaucum*, and for some of the species there was even an increase in phytotoxicity in the time between application and sowing. The *Mucuna cinerea* species, regarding the sowing time, presented a slightly greater reduction in phytotoxicity, considering the sowing period of 30 to 120 days from the application. According to Gonçalves et al. (2018) the leaching of indaziflam in both the Red-Yellow Latosol and in the cambisol was higher when the pH is high. Thus, changes in the pH of the solution at different periods, after the application of the herbicide may have altered its availability in the soil solution, which explains that in some situations there is an increase in phytotoxicity with the increase in the interval between application and sowing.

Regarding to herbicide doses, the plant *Lupinus albus*, for all sowing times, presented significant differences when compared to the control test (dose 0g a.i. $ha^{-1}$). The doses of 75 and 100g a.i. $ha^{-1}$ did not differ from each other in most treatments. Control ranged from 52.5 to 82.5%. Even considering that there was no total control of the *Lupinus albus*, it is still an important percentage of control, considering that there was no reduction in control over the sowing times.

The species *Raphanus sativus* was highly susceptible to the herbicide indaziflam, regardless of the dose used. The doses differed only at the sowing times of 90 and 120 DAA, although with a small difference of 90% to 100% control in comparison to the lowest and the highest dose, respectively. Therefore, it was not considered as potential species for use in the phytoremediation of the herbicide.

The species *Avena strigosa* was also highly susceptible to indaziflam, with the lowest phytotoxicity observed of 90% at 60 DAA at the 75g a.i. $ha^{-1}$ and with 100% control at the highest dose, at all sowing times evaluated, determining the non-selection of the species as possible phytoremediator.

The herbicide indaziflam showed high control efficacy of the species *Secale cereale* regardless of the sowing periods. There was 100% control at the highest dose in all evaluated periods, and therefore it was not selected as a species with potential to phytoremediate the herbicide indaziflam. In the USA, indaziflam has been presented as an alternative in the control of this plant, which is considered invasive in areas of wheat and barley (White et al., 2006).

The species of Crotalaria (*Crotalaria breviflora*, *Crotalaria spectabilis*, *Crotalaria juncea*) evaluated in this study were susceptible to indaziflam and with high percentages of phytotoxicity. There was no difference between the two herbicide doses, so neither of them was selected as potential phytoremediator. The results support those of Torres et al. (2018) who observed that *Crotalaria juncea* was susceptible to the herbicide indaziflam even when sowed 60 days after the application of the product.

The species *Vicia sativa* also showed high control by indaziflam, with high control percentages. The doses differed only when sowing occurred at 120 DAA, however with high phytotoxicity values, and a difference of 92.5 to 98.5% between the lowest and highest dose, respectively. Due to the high phytotoxicity presented at all sowing times and on both applied doses of the herbicide, *Vicia sativa* was not selected as potential phytoremediator.

The species *Helianthus annuus*, although it presented high control by indaziflam on its sowing at 30 DAA, at 5 DAA, it presented a phytotoxicity of 42.5 and 60.0% for the lowest (75g a.i. $ha^{-1}$) and higher dose (100g a.i. $ha^{-1}$), respectively. The results support those of Torres et al. (2018) who found that even when sowing the *Helianthus annuus* at 60 days after the application of indaziflam, the herbicide was highly phytotoxic for the crop.

The species *Canavalis ensiformis* presented low control values by the herbicide indaziflam which ranged from 26.2% to 62.5%. The results support those observed by Monquero et al. (2013). The highest control percentages were verified in the sowings with the longest interval between application and sowing of the species. Madalão et al. (2013) confirm the high performance of this species for phytoremediation of the herbicide sulfentrazone. The use of species with the potential to phytoremediate more than one herbicide would be an important advantage.

The species *Mucuna cinerea* also presented potential use for phytoremediation of indaziflam, being observed that in some treatments low control values, and no difference was found between doses (100 and 75g a.i. $ha^{-1}$), except in sowing at 5 DAA. The data support the findings of Monquero et al. (2013).

The green manure *Fagopyrum esculentum* revealed statistical difference between the doses (100 and 75g a.i. $ha^{-1}$) only in relation to sowing at 5DAA, however, in any case, it was presented a high phytotoxicity at all sowing times. Field experiments over a period of 7 years have shown that F. esculentum has acceptable tolerance to many herbicides that are known to selectively control grass weeds in broadleaf crops (Friesen and Campbell,1986), however the same cannot be mentioned about the use of indaziflam.

It was determined that the species *Sorghum bicolor* presented elevated phytotoxicity to the herbicide indaziflam, therefore this species was not selected. There was no statistical difference between doses (100 and 75g a.i. $ha^{-1}$) at any sowing time evaluated. Gonçalves et al. (2021) used *S. bicolor* plant as a bioindicator on the tests of adsorption and desorption of indaziflam in Brazilian soils with different pHs, thus ratifying the sensitivity of this species to the herbicide evaluated.

The grass species *Pennisetum glaucum* was also susceptible and no differences between the two doses evaluated were found. High control percentages were verified in most treatments; therefore, it was not selected as a potential phytoremediator.

The phytotoxicity results for most species confirm the high persistence of the herbicide. According to Guerra et al. (2016) the persistence of biological activity of indaziflam was superior to 150 days.

In the phytoremediation of herbicides, the plants generally perform in two ways, through phytodegradation in which there is the absorption and accumulation or metabolization of the product in plant tissues, degrading it or transforming it into low-toxicity compounds or through Phyto stimulation resulting from the action of rhizosphere microbes (Mello, 2021). According to Reis et al. (2021), plants that show a certain level of tolerance to herbicides commonly act by stopping the product, compartmentalizing, and metabolizing it.

The results regarding the height (cm) and reduction of dry mass of the aerial part (%) in comparison to the control (0g a.i. $ha^{-1}$) of the species of green manure compared to the height and dry mass of the green manure crops are presented in Table 2S.

The species *Lupinus albus*, showed no statistical differences in the dry mass between the evaluated doses of indaziflam (75 and 100g a.i. $ha^{-1}$), presenting steep reductions. Plant height did not differ statistically from the control (dose 0g a.i. $ha^{-1}$) at 30 and 120 DAA. The similarity of data with the control can be explained because the plant may still be upright, but it presented phytotoxicity.

For the species *Raphanus sativus*, regardless of the dose, it is possible to verify a high percentage of reduction in the dry mass of the plants, with virtually total control in most treatments, and the height of the plants presented low or zero values.

For the species *Avena strigosa* and *Secale cereale*, there was also a high negative impact of the herbicide on the dry mass, with a 100% reduction in most treatments. Plant height was generally low or nil. There are statistical differences in height for all doses when compared to the control (dose 0g a.i. $ha^{-1}$).

For the species *Crotalaria breviflora*, *Crotalaria spectabilis* and *Crotalaria juncea*, the reductions in dry mass close to 100% were also observed in comparison to the control, and no differences were observed between the applied doses of indaziflam (75 and 100g a.i. $ha^{-1}$), in agreement with the phytotoxicity data obtained for these green manure crops. The heights of the plants, in the two doses of the herbicide, differed statistically from the control (dose 0g a.i. $ha^{-1}$) of the respective species. Sebastian et al. (2016) reports on the high spectrum of control of indaziflam on both broadleaf and annual grass species, which is reflected in this study in regard to green manure.

There was a high reduction in dry mass of the species *Vicia sativa*, when compared to the control, with practically total control of the species by the herbicide. Regarding plant height, the two doses (75 and 100g a.i. $ha^{-1}$) presented significant difference when compared to the control, with high negative impact.

For the species *Helianthus annuus,* the reduction of dry mass was relatively moderated at 5DAA for both doses, with reduction of less than 25%, however at other periods there was a high reduction in dry mass, reaching 100% control when sowing at 120 DAA. In relation to the height, at 30 and 90 DAA, for the lowest dose of the herbicide (75g a.i. $ha^{-1}$) there was no difference to the control, however at the highest dose regardless of the time, there was a reduction in plant height. The results support those of Dan et al. (2012) who found that sunflower (*Helianthus annuus*) culture showed high sensitivity to residual activity of diclosulam when cultivated after soybean. Residual activity of diclosulam was detected throughout the sunflower cultivation, extending for 200 days.

The species *Canavalis ensiformis* and *Mucuna cinerea* showed low reductions in dry mass, however, there was a lower impact of the herbicide on the height of *Canavalis ensiformis* since at several times, this variable did not differ from the control. The species *Mucuna cinerea* under the effect of the herbicide, regardless of the dose, presented a reduction in plant height when compared to the control.

The species *Fagopyrum esculentum* also showed high herbicide effect on the dry mass reduction. The lowest reductions occurred at 5 and 30 DAA (71.39% and 71.07% respectively) at the lowest dose. None of the applied doses (75 and 100g a.i. $ha^{-1}$) presented statistical differences when compared to each other. In the evaluation of height, with the exception of the treatment at 30 DAA at the lowest dose, it differed from the control, with a large reduction in height, especially at the highest dose.

For the species *Sorghum bicolor* and *Pennisetum glaucum* grasses, a high reduction in dry mass was observed in comparison to the control without the herbicide, and there was no difference between the two herbicide doses for both species. There was variance in the response of the plants regarding the height of the plants depending on the period,

**Table 1.** Chemical parameters for fertility purposes of sample (0-20 cm) of Dystrophic Red Latosol.

| Dystrophic Red Latosol | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| P Resin | O.M. | pH | K | Ca | Mg | H+Al | BS | CEC | V |
| $mg/dm^3$ | $g/dm^3$ | $Ca\,Cl_2$ | | | | $mmol_c/dm^3$ | | | % |
| 15 | 38 | 5.6 | 5.4 | 53 | 13 | 26 | 71.4 | 97.4 | 73 |

**Table 2.** Phytotoxicity (%) of the herbicide indaziflam in the bioindicator *Phaseolus vulgaris*, evaluated at 50 days after emergence, after the cultivation of *Lupinus albus*, *Canavalis ensiformis* and *Mucuna cinerea* at different periods after the application (DAA) of the herbicide.

| Phytotoxicity (%) | | | | | |
|---|---|---|---|---|---|
| *Phaseolus vulgaris* after *Lupinus albus* | | | | | |
| Indaziflam (g a.i. $ha^{-1}$) | Sowing periods | | | | |
| | 5DAA | 30DAA | 60DAA | 90DAA | 120DAA |
| 0 | 0.00 bA | 0.00 bA | 0.00 cA | 0.00 bA | 0.00 bA |
| 75 | 100.00 aA | 96.25 aA | 85.00 bA | 99.50 aA | 96.25 aA |
| 100 | 100.00 aA | 96.25 aA | 97.50 aA | 93.75 aA | 92.50 aA |
| F | A=1.57ns B=1791.21** AxB=1.83ns | | | | |
| *Phaseolus vulgaris* after *Canavalis ensiformis* | | | | | |
| Indaziflam (g a.i. $ha^{-1}$) | Sowing periods | | | | |
| | 5DAA | 30DAA | 60DAA | 90DAA | 120DAA |
| 0 | 0.00 bA | 0.00 bA | 0.00 bA | 0.00 bA | 0.00 bA |
| 75 | 86.25 aA | 86.25 aA | 100.00 aA | 96.25 aA | 98.25 aA |
| 100 | 97.00 aA | 98.75 aA | 100.00 aA | 99.50 aA | 100.00 aA |
| F | A=2.99ns B=2296.40** AxB=1.91ns | | | | |
| *Phaseolus vulgaris* after *Mucuna cinerea* | | | | | |
| Indaziflam (g a.i. $ha^{-1}$) | Sowing periods | | | | |
| | 5DAA | 30DAA | 60DAA | 90DAA | 120DAA |
| 0 | 0.00 bA | 0.00 bA | 0.00 bA | 0.00 bA | 0.00 bA |
| 75 | 100.00 aA | 85.00 aA | 100.00 aA | 93.75 aA | 98.75 aA |
| 100 | 92.50 aA | 100.00 aA | 100.00 aA | 91.25 aA | 93.75 aA |
| F | A=1.03ns B=1189.60** AxB=1.79ns | | | | |

DAA: days after application. a.i.: active ingredient. Factor A: Doses; Factor B: Sowing Periods. ** significant and $^{NS}$ not significant at the 1%** probability level according to the f-test; Averages followed by equal lowercase letters in the column and uppercase in the row do not differ from each other according to the Scott-Knott test at 5% significance.

being verified a large reduction at some periods while there was no difference to the control at others. However, compared to the high phytotoxicity values observed at all times, it can be observed that the plant has often reached a certain height, but is intoxicated by the effect of the herbicide. Sebastian et al. (2017) highlights the suppressive effect of the herbicide on grasses. According to the authors, the high activity of indaziflam in monocotyledons is a management alternative for the long-term control of multiple annual invasive winter grasses and can potentially be used to eliminate the soil seed bank of these grasses.

Considering the results of phytotoxicity (Table 1S) and plant biometrics (Table 2S), the species *Lupinus albus*, *Canavalis ensiformis* and *Mucuna cinerea* were selected for evaluation of the phytoremediation of the herbicide indaziflam, in the second part of the research using the *Phaseolus vulgaris* as a bioindicator.

***Efficiency of the species selected in phytoremediation of indaziflam***

Dias et al. (2019) found that the following monocotyledons in the order: brachiaria grass, rice, corn, wheat, oats and the eudicotyledons: tomato, cucumber, sunflower, beans, soybeans have potential to be used as a bioindicator in studies with indaziflam.

The phytotoxicity results presented by the bioindicator species *Phaseolus vulgaris* at 50 DAE, sown after the cultivation of the selected green manure with and without herbicide doses application, according to the sowing times, are presented in Table 2.

The sowing of the bioindicator *Phaseolus vulgaris*, after the cultivation of the species *Lupinus albus*, regardless of the period after the indaziflam application, showed high phytotoxicity. There was no significant difference between the doses except at 60DAA, and for the lowest dose of

**Table 3.** Height (cm) and aerial dry mass (%) in relation to the control, of the bioindicator *Phaseolus vulgaris*, evaluated at 50 days after emergence, after cultivation of *Lupinus albus*, *Canavalis ensiformis* and *Mucuna cinerea* at different periods after the application of the herbicide (DAA) indaziflam.

| *Phaseolus vulgaris* after *Lupinus albus* | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Indaziflam (g a.i. $ha^{-1}$) | Sowing periods | | | | | | | | | |
| | 5DAA | | 30DAA | | 60DAA | | 90DAA | | 120DAA | |
| | Height | Dry M. | Height | Dry M. | Height | Dry M. | Height | Dry M. | Height | Dry M. |
| 0 | 14.67 a | - | 14.67 a | - | 14.67 a | - | 14.67 a | - | 14.67 a | - |
| 75 | 0.00 b | 100.00 a | 1.62 b | 99.04 a | 7.37 b | 78.88 a | 2.75 b | 98.93 a | 3.75 a | 92.55 a |
| 100 | 0.00 b | 100.00 a | 0.75 b | 97.18 a | 1.75 b | 98.29 a | 8.25 b | 92.66 a | 9.87 a | 78.97 a |
| F | 1192.28** | - | 160.96** | 0.42 ns | 17.04** | 1.65 ns | 11.48** | 0.76ns | 1.92 ns | 0.93 ns |

| *Phaseolus vulgaris* after *Canavalis ensiformis* | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Indaziflam (g a.i. $ha^{-1}$) | Sowing periods | | | | | | | | | |
| | 5DAA | | 30DAA | | 60DAA | | 90DAA | | 120DAA | |
| | Height | Dry M. | Height | Dry M. | Height | Dry M. | Height | Dry M. | Height | Dry M. |
| 0 | 22.50 a | - | 22.50 a | - | 22.50 a | - | 22.50 a | - | 22.50 a | - |
| 75 | 10.00 b | 82.10 a | 7.25 b | 88.79 a | 0.00 b | 100.00 a | 4.87 b | 83.92 a | 3.37 b | 96.27 a |
| 100 | 3.75 b | 98.36 a | 1.37 b | 99.77 a | 0.00 b | 100.00 a | 1.75 b | 98.31 a | 0.00 b | 100.00 a |
| F | 17.29** | 4.54 ns | 15.83** | 1.40 ns | 467.31** | - | 51.24** | 4.20 ns | 90.32** | 2.14 ns |

| *Phaseolus vulgaris* after *Mucuna cinerea* | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Indaziflam (g a.i. $ha^{-1}$) | Sowing periods | | | | | | | | | |
| | 5DAA | | 30DAA | | 60DAA | | 90DAA | | 120DAA | |
| | Height | Dry M. | Height | Dry M. | Height | Dry M. | Height | Dry M. | Height | Dry M. |
| 0 | 25.00 a | - | 25.00 a | - | 25.00 a | - | 25.00 a | - | 25.00 a | - |
| 75 | 0.00 b | 100.00 a | 6.87 b | 86.55 a | 0.00 b | 100.00 a | 10.00 b | 92.46 a | 2.25 b | 97.24 a |
| 100 | 4.00 b | 90.94 a | 0.00 b | 100.00 a | 1.25 b | 96.12 a | 5.25 b | 93.94 a | 3.87 b | 89.20 a |
| F | 42.71** | 1.69 ns | 19.30** | 1.46 ns | 68.20** | 1.00 ns | 18.50** | 0.12 ns | 17.74** | 0.52 ns |

DAA: days after application. Dry M.: dry mass. a.i. active ingredient. Factor A: Doses; Factor B: Sowing Periods. ** significant and $^{NS}$ not significant at the 1%** probability level according to the f-test; Averages followed by equal lowercase letters in the column and uppercase in the row do not differ from each other according to the Scott-Knott test at 5% significance.

indaziflam the bioindicator species presented the lowest level of phytotoxicity (85%), still being overly sensitive to the effects of the herbicide present in the soil.

Regarding the species *Canavalis ensiformis*, the bioindicator plants of *Phaseolus vulgaris* did not present differences between the periods at any applied dose. The doses of 75 and 100g a.i. $ha^{-1}$ did not differ from each other, and a high percentage of phytotoxicity was verified, close to 100% in most treatments. The species *Canavalis ensiformis* was effective in its phytoremediation of the herbicide trifloxysulfuron-sodium, while using the same bioindicator *Phaseolus vulgaris*; however, the assay was performed in the field (Santos et al., 2006).

For the bioindicator plant, cultivated after the species *Mucuna cinerea*, high phytotoxicity values were verified. There was no difference between the periods, and the two herbicide doses evaluated (75 and 100g a.i. $ha^{-1}$) also presented suppressive effect on the bioindicator.

Table 3 shows the results obtained regarding the evaluation of height (cm) and reduction of aerial dry mass (%) in relation to the control (0g a.i. $ha^{-1}$) of the *Phaseolus vulgaris* bioindicator performed at 50 DAE, at different sowing periods and doses of indaziflam.

The cultivation of *P. vulgaris* after the green manure *Lupinus albus* showed a reduction in dry mass ranging from 78.9% to 100%. The height of the plant did not differ from the control plant when sowing at 120DAA for the two doses applied, however as previously mentioned, due to the high phytotoxicity observed, in this case, the plant is upright, but practically dead.

Considering the biometric evaluations, it was also verified that the species *Canavalis ensiformis* was not effective in the phytoremediation of indaziflam at any of the evaluated times. The species *P. vulgaris* presented a high reduction in dry mass with values ranging from 82.1% to 100.0%. The height also presented a reduction in comparison to the control. There was no difference between the two doses of indaziflam, both of which differed from the control. Results obtained by Ferraço et al. (2017) showed efficiency of this species in phytoremediate the herbicide sulfentrazone, however, according to the authors, the minimum population density of *Canavalis ensiformis* that allowed for the development of the bioindicator plant (millet), was of 10 plants $m^{-2}$. However, better results were obtained with a density of 40 plants $m^{-2}$. Perhaps the increase in plant density can be an alternative for the viability of the species as a phytoremediator.

The species *Mucuna cinerea* also did not present efficacy as a phytoremediator of indaziflam, considering the high reduction of dry mass and height of the bioindicator *Phaseolus vulgaris*. There was no significant difference between indaziflam doses, regardless of the sowing time after the application. The results show high persistence of indaziflam in the soil, and the suppressive effects were noted even when the species of green manure are established for 120 days after the application. Although the selected potential plants have no effect on the phytoremediation of indaziflam, other means can be studied with the respective species such as the cultivation of longer cycles, the use of higher plant density or the joint use of softeners may favor a better result in phytoremediation of this herbicide.

**Material and methods**

The experiments were based on the methodologies proposed by Pires et al. (2005), Monquero et al. (2013), and Galon et al. (2017), who used bioindicator plants as a tool to evaluate phytoremediation. The experiments were conducted in a greenhouse at the Federal University of São Carlos, Center for Agricultural Sciences (CCA), Araras, São Paulo, Brazil.

***Selection of plants with potential for phytoremediation of soils contaminated by the herbicide indaziflam***

***Experiment design and plant materials***

For the selection of species with phytoremediator potential for indaziflam, 15 species of green manure were evaluated. The choice of species was made based on information related to agricultural/economic value and its use in the Central-South region, alongside with the company "Piraí Sementes." The species used were: *Lupinus albus*, *Raphanus sativus*, *Avena strigosa*, *Secale cereale*, *Crotalaria breviflora*, *Crotalaria spectabilis*, *Crotalaria juncea*, *Vicia sativa*, *Lolium multiflorum*, *Helianthus annuus*, *Canavalis ensiformis*, *Mucuna cinerea*, *Fagopyrum esculentum*, *Sorghum bicolor*, *Pennisetum glaucum*.

The trials were conducted in pots, using soil samples classified as Dystrophic Red Latosol (Claessen, 1997), in the 0-20 cm layer. Soil collection was conducted in an area with no history of herbicide use. Chemical analyses (Table 1) were performed by the Soil Chemistry and Fertility Laboratory, of the Federal University of São Carlos, Agricultural Science Center (CCA).

The experiments were carried out separately for each species of green manure, divided into three parts, considering the indicated planting times, the first part being performed with the winter green manure *Avena strigosa*, *Raphanus sativus* and *Lupinus albus*, and the herbicide application on 08/08/2021; during the second period the species *Secale cereale*, *Crotalaria juncea*, *Crotalaria breviflora*, *Crotalaria spectabilis* were evaluated, with herbicide application on 10/01/2021; and the third part evaluated the species *Vicia sativa*, *Lolium multiflorum*, *Helianthus annuus*, *Pennisetum glaucum*, *Sorghum bicolor*, *Fagopyrum esculentum*, *Mucuna cinerea*, *Canavalis ensiformis* with the application of indaziflam on 11/09/2021.

The test was completely randomized, with four replications, and the treatments were arranged in a 3 x 5 factorial layout, consisting of three doses of the herbicide indaziflam (0, 75 and 100 g i.e. $ha^{-1}$) and five sowing times after the application (5, 30, 60, 90 and 120 days).

***Herbicides application and evaluations***

The experimental unit was composed of a polyethylene vessel with volumetric capacity of 5 L, lined with a plastic bag to prevent herbicide leaching. After filling the vessels, the applications of the herbicide indaziflam (0, 75 and 100g a.i. $ha^{-1}$) were performed during preemergence. For the application, a costal spray pressurized with $CO_2$ was used, equipped with a spray bar with two Teejet 110.02 fan nozzles and using an application volume of 200 L $ha^{-1}$.

The green manure species were sown according to the predetermined times, at 5, 30, 60, 90 and 120 days after application (DAA) of the different herbicide doses. The pots were maintained with adequate moisture for plant growth.

At 60 days after emergence (DAE) of the green manure species, visual evaluations of phytotoxicity were performed in comparison to the control (zero dose of indaziflam), based on the scale proposed by the Velini et al. (1995). The scores for phytotoxicity were established as a percentage, with a score of 0 (zero) for the absence of injuries and 100 (one hundred) for the plant's death. At the same time, the height of the plants was measured from the neck to the apical meristem in the dicotyledons and to the end of the last leaf in the other plants. At 60 DAE, dry biomass of the aerial part was determined by collecting the plant and subsequent drying in a forced air circulation oven (70 ± 2 ºC) for 72 hours.

The data on the dry biomass in percentage (%) in relation to the control plant without herbicide application (0 g i.e. $ha^{-1}$) was determined by the following formula:

$$X\,(\%) = 100 - \left[\left(\frac{m\ rep\ trat\ x\ 100}{m\ \bar{x}\ test}\right)\right] (1)$$

In which,

X= percentual reduction of the treatment.

m = mass (g).

trat= treatment.

$\overline{X}$ = mean.

Test = control.

Green manure species that present phytotoxicity lower than 80% at least one sowing time were selected as potential phytoremediator.

***Efficiency of the species selected in the phytoremediation of the herbicide indaziflam using Phaseolus vulgaris as a bioindicator***

***Bioindicator seeding and evaluations***

For the selection of potential species, it was established as a criterion, a percentage of phytotoxicity lower than 80%. At each predetermined period, after the herbicide application (5, 30, 60, 90 and 120 days after the application of the different herbicide doses), the species *Phaseolus vulgaris*, sensitive to indaziflam (Dias et al., 2019), was sowed with density of 5 seeds per experimental unit.

At 15, 30 and 50 days after the emergence of the bioindicator (DAE), visual phytotoxicity was evaluated, based on the scale proposed by the Velini et al. (1995). The scores for phytotoxicity were given in percentage: 0 (zero) = absence of injuries and 100 (one hundred) = plant's death. At 50 DAE, the height of the plants measured from the neck to the apical meristem and the dry mass of the aerial part of the plants were also evaluated, the data was obtained by drying it in a forced air circulation oven (70 ±2 ºC) for 72 hours, and the dry biomass data in percentage (%) in relation to the control without herbicide application was used (formula 1).

***Statistical analysis***

The data obtained were submitted to analysis of variance by the F test. The averages were compared through the Scott-Knott test with 5% significance, using the computational statistical software ASSISTAT (Silva 2008).

**Conclusion**

The species of green manure *Raphanus sativus*, *Avena strigosa*, *Secale cereale*, *Crotalaria breviflora*, *Crotalaria spectabilis*, *Crotalaria juncea*, *Vicia sativa*, *Lolium multiflorum*, *Helianthus annuus*, *Fagopyrum esculentum*, *Sorghum bicolor* and *Pennisetum glaucum* presented elevated levels of control by the herbicide indaziflam up to 120 days after the application.

- *Lupinus albus*, *Canavalis ensiformis* and *Mucuna cinerea* were selected as potential phytoremediator as they presented lower level of control by the herbicide indaziflam but were not effective in their phytoremediation in neither of the two doses (75 and 100g a.i. $ha^{-1}$), even after 120 days of the herbicide application.

**Acknowledgment**

Thanks for the grant 2020/12985-5, to the São Paulo Research Foundation (FAPESP), for providing the resources for the development of this study and to the members of the Study Group on Agricultural Sciences (GECA) for their collaboration.

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