**Running title:** Herbicide-Resistant Fleabane with Rapid Necrosis Mechanism

**Mapping of Herbicide-Resistant Fleabane (*Conyza* spp.) and In-Depth Assessment of the Rapid Necrosis Mechanism**

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**Novelty statement**

The dose-response curve confirmed multiple resistance to diquat and 2,4-D in a Sumatran fleabane accession collected in PR. Glyphosate resistance was found in all fleabane accessions except one. Diquat resistance was found in 28 fleabane accessions. Rapid necrosis symptoms were observed in 44 fleabane accessions for 2,4-D. No rapid necrosis or any indication of resistance was observed for dicamba and triclopyr, classifying them as options for fleabane control.

**Abstract**

The aim of this study was to identify and map populations of herbicide-resistant fleabane and determine the mechanism of resistance involving rapid necrosis after the application of synthetic auxins. Seeds were collected from 60 fleabane accessions in the states of Paraná (PR) and Mato Grosso do Sul (MS), Brazil. Diquat, glufosinate, saflufenacil, glyphosate, 2,4-D, triclopyr, and dicamba were applied. Weed control was assessed 28 days after application (DAA), with accessions showing <80% control considered possibly resistant. For synthetic auxins, control was also assessed 24 hours after application (HAA) to check for rapid necrosis, confirmed for accessions with >15% control. The dose-response curve showed an accession, identified as Sumatran fleabane and collected in PR, with multiple resistance to diquat and 2,4-D. Glyphosate resistance was found in all fleabane accessions, except one. Twenty-eight accessions were resistant to diquat. For glufosinate and saflufenacil, none of the accessions were classified as resistant. Rapid necrosis was clearly observed in 44 fleabane accessions after 2,4-D application in both states, indicating the presence of the resistance mechanism. No rapid necrosis was observed for dicamba and triclopyr, or any indication of resistance.

**Keywords:** Diquat; 2,4-D; Monitoring; Weed control

**Introduction**

The genus *Conyza* (syn.: *Erigeron*) belongs to the family Asteraceae and contains around 150 species worldwide (Flann, 2016). Among the weed species, *Conyza bonariensis* (L.) Cronquist (hairy fleabane), *Conyza sumatrensis* (Retz.) E. Walker (Sumatran fleabane), and *Conyza canadensis* (L.) Cronquist (horseweed) stand out. *Conyza* spp. (fleabane) is widely distributed throughout Brazil (Lucio *et al*. 2019; Mendes *et al.* 2021). Sumatran fleabane is prevalent in Brazil, while hairy fleabane is also observed, especially in the southern region (Marochio *et al.* 2017; Ruiz *et al.* 2022).

In Brazil, cases of resistance in Sumatran fleabane to paraquat (Zobiole *et al.* 2019), 2,4-D (Queiroz et al., 2020), glyphosate, chlorimuron, and saflufenacil (Heap, 2024) have been reported. Multiple resistance to chlorimuron and glyphosate (Santos *et al.* 2014), chlorimuron, glyphosate, and paraquat (Albrecht *et al.* 2020a), as well as 2,4-D, diuron, glyphosate, paraquat, and saflufenacil (Pinho *et al.* 2019), glyphosate, chlorimuron, paraquat and 2,4-D (Lorenzetti *et al*. 2024) has also been observed. There has been a significant increase in control failures and confirmed cases of resistance to 2,4-D. Resistance to this herbicide is associated with the rapid necrosis mechanism (Queiroz *et al.* 2020; Angonese *et al.* 2023).

Rapid necrosis involves cell death due to the accumulation of reactive oxygen species (ROS), with subsequent recovery of resistant plants. Fleabane plants exhibit leaf burning approximately 2 hours after application, followed by regrowth of axillary and apical buds (Queiroz *et al.* 2020). Plant recovery usually occurs one to two weeks after application (Leal *et al.* 2022), disrupting sequential applications with desiccant herbicides.

Rapid necrosis is a response distinct from the common symptoms observed in susceptible plants after the application of synthetic auxins, where epinasty, leaf curling, and other leaf abnormalities are common. The death of susceptible plants occurs slowly, typically between 3 and 5 weeks after application (Grossmann, 2010; Peterson *et al.* 2016).

Similar cases can be found in the literature, such as the rapid response of *Ambrosia trifida* (giant ragweed) to glyphosate application. The leaves exhibit symptoms similar to contact herbicide application and detach from the plant in less than 24 hours after application, with no translocation of the herbicide to other parts of the plant (Van Horn *et al.* 2018).

Monitoring makes it possible to identify the evolution and spread of resistance cases, providing important information for effective weed management (Ye *et al.* 2016; Albrecht *et al.* 2020b; Mendes *et al.* 2021). Monitoring weed resistance cases is, therefore, an essential practice to understand, identify, and quantify the frequency of these resistant plants beforehand (Schultz *et al.* 2015). Thus, resistance monitoring studies lead to increased research and the development of new techniques to control problematic plants, aimed at decreasing selection pressure (Bunchek *et al.* 2020; Busi *et al.* 2020).

In this respect, mapping fleabane populations makes it possible to identify the evolution and spread of resistance cases, providing important information for effective control decision-making. As such, the aim of the present study was to identify and map herbicide-resistant fleabane populations and determine the resistance mechanism of rapid necrosis after the application of synthetic auxins.

**Materials and Methods**

**Seed collection and screening**

Seeds were collected in 2020 and 2021 in the states of Mato Grosso do Sul (MS) and Paraná (PR). Collections followed the methodology proposed by Burgos *et al*. (2013), where seeds were extracted from one or more plants with similar characteristics at targeted points of control failure after herbicide application. Some samples were also collected in areas with little herbicide use, in order to find susceptible plants. For each location, seeds were collected from 5 to 10 plants with the same characteristics, grouped into a single sample per location (with at least 1,000 physiologically mature seeds per sample). Field materials were cleaned and stored in a refrigerated environment.

The experiment was conducted in a greenhouse under controlled temperature (25 °C), irrigation of 5 mm day-1, and a 12-hour photoperiod. The seeds collected from each population were sown in 0.8 L plastic pots filled with germination substrate. The seedlings were transplanted after germination, with the same characteristics as their previous counterparts, with two plants per pot. The plants showed no signs of transplantation stress.

A completely randomized design with 8 treatments and 4 replications was used. The treatments consisted of the following herbicide applications: diquat (Reglone®, 400 g active ingredient [ai] ha-1) + adjuvant (Agral®, 2 L ha-1), saflufenacil (Heat®, 35 g ai ha-1) + adjuvant composed of soybean methyl ester (Mees™, 0.5 L ha-1), glufosinate (Finale®, 500 g ai ha-1) + adjuvant composed of soybean methyl ester (0.5 L ha-1), 2,4-D (Aminol® 806, 1,005 g acid equivalent [ae] ha-1), triclopyr (Triclon®, 960 g ae ha-1) + mineral oil (Lanzar®, 0.5 L ha-1), dicamba (Atectra®, 480 g ae ha-1) + adjuvant composed of soybean methyl ester (0.5 L ha-1), glyphosate (Roundup Ready®, 1,200 g ae ha-1), and a control with no herbicide application. The doses selected were within the recommended range (Rodrigues & Almeida, 2018), according to common usage by farmers in the region. Application occurred at the 6-leaf stage, using a pressurized CO2 sprayer at a constant pressure of 2 kgf cm-2, with an AIXR 110.015 four-nozzle flat-fan boom (TeeJet Technologies, Wheaton, IL), placed 0.50 m from the target and at a speed of 1 m s-1, providing a total spray volume of 150 L ha-1.

Control was assessed at 28 days after application (DAA), assigning visual scores to each experimental unit, where 0 represents no damage and 100% plant death (Velini *et al.* 1995). Control scores were used to classify the samples, considering those with values <80% as possibly resistant.

Control was also assessed 24 hours after application (HAA) of synthetic auxins to check for rapid necrosis symptoms. Rapid necrosis is characterized by leaf necrosis around 2 HAA, and subsequently, plants exhibit regrowth in axillary and apical buds (Queiroz *et al.* 2020). In addition to observing this symptomatology, it was determined that plants with scores >15% at 24 HAA displayed rapid necrosis, due to the visual intensity of symptom progression, given that typical symptoms of synthetic auxin application in susceptible plants take longer to appear and are less noticeable at 24 HAA.

**Dose-response curve**

A dose-response curve (F2 generation) was performed with an accession (B19), identified as Sumatran fleabane, and collected in Palotina - PR (24°22'59.0"S 53°42'48.5"W), which was classified as possibly resistant (<80% control), the susceptible accession (B14) was collected in Itaquiraí - MS (23°22'15.6"S 54°05'22.9"W). The same screening methodology was followed for sowing and transplanting under the same conditions of location, environment, pots, substrate, design, and number of replications. Doses of 0; 50; 100; 200; 400; 800; 1,600; 3,200 g ai ha -1 of diquat and 0; 125; 251; 502; 1,005; 2,010; 4,020 and 8,040 g ae ha -1 of 2,4-D were used. The doses used for each herbicide corresponded to 0, 1/8x, 1/4x, 1/2x, 1x, 2x, 4x and 8x the dose used in screening.

Control was evaluated at 28 DAA according to the same methodology used in screening. The data were subjected to regression analysis (p < 0.05) and adjusted to the proposed non-linear logistic regression model (Streibig 1988).

$$y=\frac{a}{\left[1+\left(\frac{x}{b}\right)^{c}\right]}$$

The non-linear logistic model provides a dose estimate to obtain the 50% control value (C50). Therefore, we opted for the mathematical calculation using the inverse equation of Streibig (1988), allowing the calculation of C50 , as proposed by Souza *et al*. (2000). Based on the C50 values, the resistance factor (RF) was determined, which is the result of the ratio between the parameters of the resistant and susceptible biotype (Burgos, 2015; Takano *et al.* 2017).

$$x=b\left(\left|\frac{a}{y}-1\right|\right)^{\frac{1}{c}}$$

Where *y* is the response variable (percentage of control or dry mass); *x* is the herbicide dose; *a* is the range between the maximum and minimum point of the variable; *b* is the dose that provides a 50% response and *c* is the slope of the curve.

**Results**

**Mapping and identification of herbicide-resistant fleabane**

A total of 60 fleabane accessions were collected, 28 of which were classified as possibly resistant (>80% control) to diquat (Table 1). Diquat resistance was confirmed by the dose-response curve constructed for the Sumatran fleabane B19 accession (F2 generation), with a C50 value of 518.3 g ai ha-1 for the resistant accession and 41.8 g ai ha-1 for the susceptible accession, resulting in a RF of 12.4 (Figure 1).

For saflufenacil or glufosinate, no accession was classified as possibly resistant, with at least 93.5 or 94.5% control, respectively, whereas for glyphosate, the widespread distribution of fleabane resistance is evident, with only one accession not classified as possibly resistant (Table 1). The accession assessed on the dose-response curve for other herbicides would most likely be classified as resistant to glyphosate, since, in screening, control was only 2% at 28 DAA for this accession, but it was not tested due to germination failures and operational issues.

**Investigation of 2,4-D-resistant fleabane and the mechanism of rapid necrosis**

In the analysis of synthetic auxin application, rapid necrosis symptoms were observed for 2,4-D in fleabane plants. Of the 60 accessions collected, only 16 showed no rapid necrosis (<15% control) (Table 2). Accessions with rapid necrosis occur in both states (Figure 2). No rapid necrosis was observed for triclopyr and dicamba in any of the accessions (Table 2).

In control analysis at 28 DAA (Table 2), all synthetic auxins showed efficacy above 80%, indicating no resistance. This is valid for triclopyr, dicamba, and accessions where 2,4-D did not cause rapid necrosis. However, the 44 accessions with rapid necrosis at 24 HAA after 2,4-D application, identified as a mechanism of resistance to this herbicide, can be classified as possibly resistant to 2,4-D. As previously explained, control above 80% at 28 DAA may be related to the developmental stage of the plants. It is important to note that 2,4-D resistance was confirmed by the dose-response curve constructed for the Sumatran fleabane B19 accession (F2 generation), with a C50 value for the resistant accession of 1,986.6 and 699.3 g ae ha-1 for the susceptible accession, resulting in an RF of 2.8 (Figure 3), reinforcing the classification of accessions with rapid necrosis by screening as possibly resistant to 2,4-D.

**Discussion**

For glyphosate, the widespread distribution of fleabane resistance is evident, with only one accession not classified as possibly resistant. Other studies have also observed extensive distribution of glyphosate-resistant fleabane in PR and MS in recent years (Lucio *et al.* 2019; Mendes *et al.* 2021).

The use of management strategies is essential for successful fleabane control, since the cultivation system in the region and repeated use of the same herbicide have resulted in the selection of several resistance cases. However, based on the results, products such as glufosinate and saflufenacil are still excellent tools in controlling this weed. Glufosinate and saflufenacil can be applied in soybean pre-seeding, used alone, in combination with each other, or with glyphosate, and synergistic effects are observed for these mixtures in fleabane control (Waggoner *et al.* 2011; Dalazen *et al.* 2015; Takano *et al.* 2020; Albrecht *et al.* 2022a, 2023). Given that glyphosate is widely used, resistant populations are broadly distributed, but when associated with other products, it can still be a tool in fleabane control, since it can improve control when combined with other herbicides (Dalazen *et al.* 2015).

In general, diquat is not effective in fleabane control, a fact directly linked to its chemical proximity with paraquat, both derived from bipyridylium compounds (Bromilow 2004). Paraquat was formerly one of the main tools in fleabane control and, due to its widespread use, faced considerable selection pressure in resistant accessions (Zobiole *et al.* 2019). After this molecule was banned, it was replaced by diquat in pre-planting and pre-harvest desiccations (Albrecht *et al.* 2022b). However, due to their shared mechanism of action and chemical group, control failures have been observed in many locations where paraquat-resistant fleabane accessions were identified.

It is important to note that despite exhibiting the rapid necrosis resistance mechanism after 2,4-D application, young plants may not regrow and could eventually die (Angonese *et al.* 2023). The intense manifestation of rapid necrosis produces symptoms similar to those of contact herbicides, leading the young plant to death. In this study, the application was at the 6-leaf stage. The more advanced the stage, the greater the chance and speed of fleabane regrowth.

For control analysis all synthetic auxins showed efficacy above 80%, indicating no resistance. This is valid for triclopyr, dicamba, and accessions where 2,4-D did not cause rapid necrosis. However, the accessions with rapid necrosis after 2,4-D application, identified as a mechanism of resistance to this herbicide (Queiroz *et al.* 2020; Angonese *et al.* 2023), can be classified as possibly resistant to 2,4-D.

Rapid necrosis is a different response from the common symptomatology in susceptible plants after synthetic auxin application (Leal *et al.* 2022; Angonese *et al.* 2023) where epinasty, curling, and other leaf changes are common. The death of susceptible plants occurs slowly, usually between 3 and 5 weeks after application (Grossmann 2010; Peterson *et al.* 2016).

Given that populations resistant to diquat and 2,4-D are increasingly frequent, management practices must be adapted with alternatives for these herbicides in fleabane control. As observed in screening, dicamba and triclopyr are possible substitutes for 2,4-D in the first fleabane control intervention. Mixtures of dicamba, triclopyr, or halauxifen + diclosulam with glyphosate have proven to be effective in fleabane control (McCauley *et al.* 2018; Cantu *et al.* 2021; Albrecht *et al.* 2022c). It is essential to rotate products and mechanisms of action for efficient management. Necrotic spotting symptoms have been observed in triclopyr for years, but nothing has been confirmed from the experiments conducted. Nevertheless, attention should be paid due to the increased use of this herbicide, with a view to replacing 2,4-D.

Depending on the plant’s stage, fleabane management requires more than one intervention for effective control. The developmental stage can affect the efficacy of synthetic auxins, with a variation in effectiveness between different herbicides (McCauley & Young, 2019; Crose *et al.* 2020). It can also determine the control of resistant plants, as seen in the present study. In general, farmers make the first application with systemic herbicides, typically glyphosate + synthetic auxins, followed by sequential applications of contact herbicides. Herbicides such as glufosinate, saflufenacil, among others, should be incorporated into management strategies.

The use of pre-emergents is crucial in fleabane control (Garcia *et al.* 2023; Silva *et al.* 2023), and an important strategy for management and preventing the selection of new resistance cases. As such, we underscore the need for integrated management, especially rotating mechanisms of action, in order to reduce selection pressure and guarantee that the available tools last longer. Studies like this one are essential for the development of integrated management.

**Conclusions**

The dose-response curve confirmed multiple resistance to diquat (photosystem I inhibitors – Group D) and 2,4-D (synthetic auxins – Group O) in a Sumatran fleabane accession collected in PR.

Glyphosate resistance was found in all fleabane accessions except one, demonstrating widespread glyphosate-resistant fleabane in PR and MS. For diquat, 28 resistant accessions were identified. None of the accessions were classified as resistant to glufosinate and saflufenacil, characterizing them as viable options for fleabane control.

Rapid necrosis symptoms were observed in 44 fleabane accessions found in PR and MS, 24 hours after 2,4-D application. Control above 80% was observed, due to the early stage of plant development at the time of application, which does not invalidate the presence of a resistance mechanism. No rapid necrosis or any indication of resistance was observed for dicamba and triclopyr, classifying them as options for fleabane control.

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**Author Contributions**

MGC, AJPA, LPA and AAMB planned the experiments, MGC, AAB, WFL and FMB carried out the experiments and collected the data, MGC, AJPA, LPA, AFMS and AAMB interpreted the results, MGC, AFMS and AJPA wrote the original version of the manuscript and MGC, WFL, AAB and AFMS statistically analyzed the data and made illustrations. All authors reviewed and approved the final version of the manuscript.

**Conflict of Interest**

All authors declare no conflict of interest.

**Data Availability**

Data presented in this study will be available on a fair request to the corresponding author.

**Ethics Approval**

Not applicable to this paper.

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**Fig. 1**: Dose-response curve for the control of Sumatran fleabane accessions at 28 days after diquat application



**Fig. 2**: Fleabane accessions with or without indication of rapid necrosis in response to 2,4-D application



**Fig. 3:** Dose-response curve for the control of Sumatran fleabane accessions at 28 days after 2,4-D application.

**Table 1**: Fleabane control at 28 days after the application of herbicides diquat, saflufenacil, glufosinate, and glyphosate. MS and PR Brazil, 2020 and 2021

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Diquat | Saflufenacil | Glufosinate | Glyphosate |  | Diquat | Saflufenacil | Glufosinate | Glyphosate |
|  | % |  | % |
| B01 | 85.5 | 96.0 | 99.8 | 3.5 | B31 | 99.8 | 99.8 | 99.5 | 4.8 |
| B02 | 63.5 | 94.8 | 94.5 | 3.0 | B32 | 99.8 | 99.8 | 100.0 | 17.8 |
| B03 | 99.8 | 98.5 | 99.8 | 1.0 | B33 | 99.5 | 99.8 | 99.8 | 30.3 |
| B04 | 31.3 | 99.8 | 99.8 | 0.5 | B34 | 97.0 | 99.5 | 99.3 | 30.3 |
| B05 | 61.0 | 99.3 | 99.5 | 3.3 | B35 | 99.5 | 99.3 | 99.3 | 6.5 |
| B06 | 66.5 | 96.3 | 97.5 | 3.3 | B36 | 51.3 | 99.8 | 99.7 | 11.0 |
| B07 | 99.8 | 99.8 | 97.5 | 4.0 | B37 | 99.5 | 100.0 | 99.5 | 4.5 |
| B08 | 52.5 | 95.0 | 99.8 | 0.0 | B38 | 99.5 | 99.8 | 99.3 | 3.3 |
| B09 | 58.8 | 98.5 | 99.8 | 0.8 | B39 | 35.0 | 100.0 | 98.8 | 3.0 |
| B10 | 37.5 | 97.5 | 99.5 | 1.5 | B40 | 99.5 | 99.8 | 99.0 | 3.5 |
| B11 | 45.8 | 98.3 | 99.8 | 5.0 | B41 | 39.8 | 99.8 | 98.5 | 8.0 |
| B12 | 55.0 | 93.5 | 99.8 | 2.8 | B42 | 38.5 | 99.5 | 98.3 | 8.0 |
| B13 | 100.0 | 99.8 | 99.8 | 4.3 | B43 | 32.5 | 99.5 | 99.5 | 1.8 |
| B14 | 100.0 | 99.3 | 99.8 | 4.5 | B44 | 99.5 | 99.5 | 99.8 | 4.0 |
| B15 | 91.0 | 99.8 | 99.8 | 5.5 | B45 | 63.8 | 97.5 | 98.0 | 3.8 |
| B16 | 98.3 | 99.5 | 99.5 | 5.5 | B46 | 100.0 | 99.8 | 98.8 | 4.5 |
| B17 | 20.0 | 99.5 | 99.8 | 2.8 | B47 | 50.0 | 97.0 | 99.8 | 1.0 |
| B18 | 48.5 | 99.5 | 99.8 | 3.5 | B48 | 61.3 | 99.5 | 99.5 | 2.0 |
| B19 | 31.3 | 97.5 | 99.8 | 2.0 | B49 | 99.8 | 98.0 | 99.5 | 2.5 |
| B20 | 68.8 | 98.8 | 99.5 | 2.3 | B50 | 45.0 | 98.3 | 99.5 | 5.5 |
| B21 | 21.3 | 98.3 | 100.0 | 2.0 | B51 | 53.8 | 96.0 | 99.5 | 3.3 |
| B22 | 56.3 | 96.8 | 99.8 | 3.8 | B52 | 100.0 | 99.5 | 99.5 | 3.5 |
| B23 | 99.8 | 99.5 | 99.8 | 2.0 | B53 | 99.8 | 99.5 | 99.0 | 10.5 |
| B24 | 100.0 | 100.0 | 100.0 | 99.8 | B54 | 99.5 | 99.8 | 99.3 | 4.8 |
| B25 | 89.8 | 99.5 | 99.8 | 6.3 | B55 | 86.0 | 99.5 | 99.5 | 4.5 |
| B26 | 21.8 | 99.8 | 99.8 | 5.0 | B56 | 99.3 | 99.8 | 99.3 | 15.5 |
| B27 | 59.8 | 98.8 | 99.8 | 5.5 | B57 | 99.0 | 99.8 | 99.5 | 12.5 |
| B28 | 99.5 | 99.3 | 99.3 | 12.5 | B58 | 99.5 | 99.8 | 99.8 | 13.5 |
| B29 | 99.5 | 99.5 | 99.0 | 6.3 | B59 | 97.0 | 99.8 | 99.5 | 16.5 |
| B30 | 99.8 | 99.8 | 99.5 | 6.5 | B60 | 55.0 | 99.3 | 99.5 | 8.0 |

Values in red indicate possibly resistant accessions.

**Table 2**: Fleabane control at 24 hours after application (HAA) and 28 days after application (DAA) of herbicides 2,4-D, triclopyr, and dicamba. MS and PR Brazil, 2020-2021 growing season

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | 2,4-D | Triclopyr | Dicamba |  | 2,4-D | Triclopyr | Dicamba |
|  | 24 HAA | 28 DAA | 24 HAA | 28 DAA | 24 HAA | 28 DAA |  | 24 HAA | 28 DAA | 24 HAA | 28 DAA | 24 HAA | 28 DAA |
|  | % |  | % |
| B01 | 20.0 | 99.5 | 3.8 | 99.3 | 3.0 | 95.3 | B31 | 46.8 | 98.3 | 6.8 | 96.3 | 4.5 | 93.5 |
| B02 | 17.5 | 93.5 | 4.8 | 99.3 | 3.3 | 93.8 | B32 | 12.5 | 96.3 | 8.8 | 97.3 | 3.5 | 93.5 |
| B03 | 6.3 | 97.5 | 4.5 | 98.8 | 3.0 | 94.5 | B33 | 55.5 | 99.3 | 5.3 | 95.3 | 4.0 | 93.3 |
| B04 | 5.0 | 95.8 | 4.5 | 97.0 | 3.0 | 94.8 | B34 | 37.5 | 99.3 | 6.3 | 98.8 | 4.3 | 95.3 |
| B05 | 16.3 | 99.5 | 4.0 | 96.3 | 3.0 | 98.0 | B35 | 57.5 | 98.8 | 7.8 | 98.0 | 4.8 | 96.8 |
| B06 | 21.3 | 97.8 | 4.5 | 99.5 | 3.3 | 98.0 | B36 | 57.5 | 99.3 | 11.0 | 98.5 | 4.3 | 96.3 |
| B07 | 13.8 | 97.0 | 4.0 | 99.5 | 2.8 | 98.0 | B37 | 8.5 | 97.0 | 6.0 | 98.8 | 4.0 | 95.5 |
| B08 | 56.3 | 87.0 | 6.3 | 88.3 | 3.0 | 93.8 | B38 | 7.5 | 96.0 | 6.8 | 97.5 | 4.3 | 92.5 |
| B09 | 34.8 | 84.3 | 7.3 | 84.8 | 2.8 | 93.0 | B39 | 49.8 | 99.3 | 7.5 | 97.5 | 4.3 | 95.0 |
| B10 | 58.0 | 92.3 | 6.8 | 90.0 | 3.5 | 90.8 | B40 | 7.3 | 97.0 | 7.3 | 98.8 | 4.0 | 97.3 |
| B11 | 55.5 | 89.0 | 6.8 | 87.5 | 2.5 | 95.0 | B41 | 56.8 | 96.5 | 9.0 | 98.8 | 3.8 | 95.0 |
| B12 | 48.3 | 85.5 | 6.8 | 87.3 | 3.8 | 90.0 | B42 | 53.0 | 97.3 | 11.8 | 98.8 | 4.5 | 93.8 |
| B13 | 48.0 | 88.8 | 9.0 | 94.3 | 3.0 | 93.8 | B43 | 8.0 | 96.0 | 6.0 | 96.3 | 4.3 | 93.5 |
| B14 | 5.8 | 86.3 | 5.0 | 97.5 | 2.8 | 94.3 | B44 | 13.8 | 98.3 | 4.5 | 96.5 | 3.8 | 97.5 |
| B15 | 3.0 | 91.0 | 3.8 | 84.5 | 2.8 | 91.8 | B45 | 20.0 | 97.3 | 5.8 | 98.8 | 4.3 | 97.5 |
| B16 | 17.5 | 87.5 | 4.0 | 99.5 | 2.8 | 92.0 | B46 | 13.8 | 97.3 | 5.0 | 99.0 | 3.8 | 97.5 |
| B17 | 49.8 | 89.8 | 8.8 | 97.0 | 3.0 | 92.5 | B47 | 51.3 | 93.3 | 6.0 | 90.0 | 3.8 | 94.5 |
| B18 | 44.8 | 93.5 | 8.0 | 95.8 | 3.3 | 91.0 | B48 | 36.0 | 88.0 | 6.8 | 87.3 | 3.8 | 93.5 |
| B19 | 47.5 | 92.5 | 8.3 | 88.0 | 2.3 | 95.0 | B49 | 53.0 | 93.3 | 6.5 | 91.5 | 4.3 | 91.5 |
| B20 | 39.8 | 85.0 | 9.3 | 93.5 | 2.8 | 88.8 | B50 | 53.5 | 94.0 | 7.3 | 89.3 | 4.0 | 95.8 |
| B21 | 23.0 | 88.0 | 5.3 | 92.3 | 3.3 | 92.5 | B51 | 47.0 | 87.5 | 7.5 | 90.8 | 4.3 | 91.3 |
| B22 | 17.5 | 89.5 | 5.5 | 94.8 | 3.3 | 91.3 | B52 | 49.3 | 91.0 | 8.0 | 96.0 | 3.8 | 94.3 |
| B23 | 45.0 | 90.5 | 8.0 | 95.3 | 3.3 | 94.5 | B53 | 38.0 | 97.8 | 8.3 | 98.5 | 5.3 | 92.5 |
| B24 | 6.0 | 100.0 | 3.8 | 100.0 | 3.3 | 99.8 | B54 | 9.0 | 95.8 | 6.8 | 96.0 | 5.5 | 97.0 |
| B25 | 45.0 | 96.3 | 9.3 | 99.0 | 3.0 | 97.3 | B55 | 43.8 | 98.8 | 6.3 | 96.3 | 5.0 | 98.5 |
| B26 | 52.5 | 98.8 | 10.8 | 98.0 | 3.3 | 97.0 | B56 | 48.0 | 98.3 | 7.5 | 97.5 | 4.0 | 95.3 |
| B27 | 44.8 | 99.0 | 10.3 | 98.5 | 4.0 | 95.0 | B57 | 13.8 | 96.8 | 7.5 | 97.3 | 4.0 | 94.0 |
| B28 | 38.5 | 98.5 | 9.5 | 98.3 | 4.3 | 92.5 | B58 | 55.0 | 98.5 | 6.8 | 95.5 | 4.5 | 93.8 |
| B29 | 6.0 | 95.0 | 4.8 | 96.5 | 4.5 | 96.8 | B59 | 36.3 | 99.0 | 6.8 | 98.5 | 5.0 | 95.8 |
| B30 | 42.5 | 98.8 | 5.5 | 95.8 | 5.0 | 98.0 | B60 | 56.3 | 97.0 | 7.3 | 97.8 | 5.3 | 97.3 |

Values in red indicate the characterization of rapid necrosis for the herbicide 2,4-D.