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Sensitivity of *Digitaria insularis* to herbicides in agricultural areas, in the Brazilian Cerrado biome

Núbia Maria Correia[(1 ✉)],
Pedro Eduardo Rampazzo[(2)],
Lucas da Silva Araújo[(3)] and
Caio Vitagliano Santi Rossi[(2)]

(1) Embrapa Cerrados, Rodovia BR-020, Km 18, Caixa Postal 08223, CEP 73310-970 Brasília, DF, Brazil.
E-mail: nubia.correia@embrapa.br

(2) Corteva Agriscience, Rodovia SP-147, Km 71,5, CEP 13800-970 Mogi Mirim, SP, Brazil.
E-mail: pedro.rampazzo@corteva.com, caio.rossi@corteva.com

(3) Universidade de São Paulo, Escola Superior de Agricultura Luiz de Queiroz, Departamento de Produção Vegetal, Avenida Pádua Dias, nº 11, CEP 13418-900 Piracicaba, SP, Brazil.
E-mail: lucasilva_31@hotmail.com

✉ Corresponding author

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Abstract – The objective of this work was to evaluate the sensitivity of different populations of *Digitaria insularis* to the glyphosate, clethodim, and haloxyfop-P-methyl herbicides, in agricultural areas, and to develop infestation maps based on the responses of these populations. One hundred sixty-one populations suspected of being resistant were evaluated and compared to a susceptible population. When plants displayed three to four tillers, the populations were sprayed with glyphosate (1,000 g ha^{-1} a.e.), clethodim (108 g ha^{-1} a.i. + 0.5% mineral oil), and haloxyfop-P-methyl (62.35 g ha^{-1} a.i. + 0.5% mineral oil); plants without herbicide application were used as the control. The plant populations were classified as susceptible, intermediately resistant (with susceptible and resistant plants), or resistant to the tested herbicides. All populations were susceptible to clethodim; 97.5% were susceptible and 2.5% were intermediately resistant to haloxyfop-P-methyl; and 9.9% were susceptible, 21.1% intermediately resistant, and 68.9% resistant to glyphosate. Glyphosate-resistant populations are homogeneously distributed throughout the evaluated regions. There are no cases of *D. insularis* multiple resistance in the sampled regions; however, cross-resistance to glyphosate and haloxyfop-P-methyl was detected.

Index terms: clethodim, glyphosate, haloxyfop-P-methyl, sourgrass, weed.

Sensibilidade de *Digitaria insularis* a herbicidas em áreas agrícolas no bioma Cerrado brasileiro

Resumo – O objetivo deste trabalho foi avaliar a sensibilidade de diferentes populações de *Digitaria insularis* aos herbicidas glifosato, cletodim e haloxifope-P-metílico, em áreas agrícolas, e elaborar mapas de infestação com base na resposta das populações aos herbicidas. Cento e sessenta e uma populações com suspeita de serem resistentes foram avaliadas e comparadas a uma população suscetível. Quando as plantas estavam com três a quatro perfilhos, as populações foram pulverizadas com glifosato (1.000 g ha^{-1} e.a.), cletodim (108 g ha^{-1} i.a. + 0,5% de óleo mineral) e com haloxifope-P-metílico (62,35 g ha^{-1} i.a. + 0,5% de óleo mineral); plantas sem aplicação de herbicidas foram usadas como controle. As populações foram classificadas como sensíveis, resistentes ou intermediárias (com plantas sensíveis e resistentes) aos herbicidas-teste. Todas as populações foram sensíveis ao cletodim; 97,5% foram sensíveis e 2,5% intermediárias a haloxifope-P-metílico; e 9,9% foram sensíveis, 21,1% intermediárias e 68,9% resistentes ao glifosato. Populações resistentes ao herbicida glifosato estão dispersas homogeneamente nas regiões avaliadas. Não há casos de resistência múltipla de *D. insularis* nas regiões amostradas; no entanto, detectou-se resistência cruzada a glifosato e a haloxifope-P-metílico.

Termos para indexação: cletodim, glifosato, haloxifope-P-metílico, capim-amargoso, plantas daninhas.

Introduction

Digitaria insularis (L.) Fedde is a perennial, herbaceous, erect weed species that forms tufts of short rhizomes, and reproduces by seed that are covered by hairs; thus, they are carried by the wind to great distances (Kissmann & Groth, 1997). Resistant biotypes of *D. insularis* were selected in agricultural areas due to the successive and intense use of the glyphosate herbicide. In Brazil, the resistance of this species to glyphosate was first reported in 2008, in the state of Paraná (Heap, 2020). The first case of *D. insularis* resistance to acetyl-CoA carboxylase (ACCase) inhibitor of herbicides from the aryloxyphenoxypropionate group (fenoxaprop-P-ethyl and haloxyfop-P-methyl) was reported in 2016 (Heap, 2020).

The infestation of resistant biotypes of *D. insularis* to glyphosate has increased in agricultural areas in the Brazilian Cerrado biome (Lucio et al., 2019). This can result in increases of the production costs because of the need to adopt other management strategies, or in the yield reduction of the crop of economic interest due to competition with weeds that are not controlled (Gazziero et al., 2019). According to Adegas et al. (2018), the cost of weed management in soybean crop areas with glyphosate-resistant *D. insularis* may increase from 165 to 290%. Resistant *D. insularis* plants are difficult to control due to the reduced chemical control options available (Carpejani & Oliveira, 2013; Gazola et al., 2016; Zobiole et al., 2016), which not only requires changes of herbicides, but also of the weed management in agricultural areas, from the medium to long term, considering rotations and mixtures of herbicide, and the use of soil cover crops to mitigate the weed resistance and prevent the selection of biotypes with multiple resistance (Marochi et al., 2018).

Field surveys and tests to evaluate the possible resistance to herbicides are important for weed management practices (Norsworthy et al., 2012). Weed monitoring encompasses activities that confirm resistance in geographical areas defined in a single point in time, or over several years (Soteres & Peterson, 2015). In addition, the monitoring can prevent the dispersal of resistant plants and address regional issues, raising awareness of producers in each region and improving weed management (Lopez Ovejero et al., 2017).

Technical visits to farms and field observations have shown flaws in the chemical control of *D. insularis* in agricultural areas, in the states of Goiás and Minas Gerais, and in the Federal District, in Brazil, and resulted in complains of producers. The survival of *D. insularis* in the field can be an indication of resistance to glyphosate (Gazola et al., 2016; Zobiole et al., 2016; Lucio et al., 2019).

The objective of this work was to evaluate the sensitivity of different populations of *D. insularis* to the glyphosate, clethodim, and haloxyfop-P-methyl herbicides, in agricultural areas, and to develop infestation maps based on the responses of these populations.

Materials and methods

One hundred sixty-one *D. insularis* populations were evaluated and compared to a known susceptible population (standard population). Fifty-six municipalities were sampled - 33 in the state of Goiás, 22 in the state of Minas Gerais, and 1 in the Federal District of Brazil. The samples were concentrated in the south and southwest regions of Goiás, and in Triângulo Mineiro, Alto Paranaíba, and northwest regions of Minas Gerais (Table 1).

Most populations (158) were from agricultural areas with summer soybean crops. Out of these, 66 were maintained with weeds in the off-season (fallow), and the rest with crops in the autumn-winter season in the following number of areas: beans (7), vegetables (4), and wheat (3), irrigated by center pivot; and cotton (2), maize (53), millet (11), and sorghum (12), under rainfed conditions. The other two populations were from agricultural areas with coffee cultivation.

Twenty to forty panicles were collected from the largest number of plants possible in infestation spots, in each crop field (sample unit). The panicles were collected when seeds were at the stage in which they were easily detachable from the panicle. The panicles collected in the sampling units were packed in paper bags and identified with the property name, municipality, state, geographic coordinates (latitude and longitude), altitude, and crop history of the last two crop seasons.

The seed samples were sent to the sector of Weed Sciences of Embrapa Hortaliças, in Brasília, DF, Brazil, where the study was developed. The sensitivity

Table 1. Municipality, state, geographic coordinates, altitude, and crops of the agriculture areas of each population (Pop.) of *Digitaria insularis*.

Pop.	Municipality	State(1)	Geographic coordinates	Altitude (m)	Crop (summer - off-season)
1	Lagoa Grande	MG	17º76'05" S, 46º53'73" W	557	Soybean-fallow
2	Monte Alegre	MG	19º00'85" S, 48º58'77" W	702	Soybean-fallow
3	Uberlândia	MG	18º51'42" S, 48º26'40" W	828	Soybean-fallow
4	Uberlândia	MG	18º50'89" S, 48º25'65" W	833	Soybean-fallow
5	Padre Bernardo	GO	15º19'00" S, 48º22'30" W	700	Soybean-fallow
6	Tupaciguara	MG	18º35'04" S, 48º51'39" W	877	Soybean-fallow
7	Tupaciguara	MG	18º35'66" S, 48º53'01" W	820	Soybean-fallow
8	Buritis	MG	15º43'77" S, 46º24'98" W	919	Soybean-fallow
9	Buritis	MG	15º43'26" S, 46º25'11" W	920	Soybean-corn
10	Buritis	MG	15º29'79" S, 46º29'79" W	922	Soybean-corn
11	Buritis	MG	15º48'03" S, 46º25'51" W	914	Soybean-corn
12	Buritis	MG	15º42'59" S, 46º21'32" W	907	Soybean-corn
13	Chapada Gaúcha	MG	15º19'05" S, 45º35'47" W	835	Soybean-fallow
14	Água Fria de Goiás	GO	14º48'94" S, 47º39'92" W	1198	Soybean-fallow
15	São João da Aliança	GO	14º42'60" S, 47º35'50" W	1199	Soybean-fallow
16	Padre Bernardo	GO	15º24'51" S, 48º26'34" W	668	Soybean-fallow
17	Padre Bernardo	GO	15º21'45" S, 48º26'33" W	755	Soybean-fallow
18	Padre Bernardo	GO	15º20'00" S, 48º25'13" W	750	Soybean-fallow
19	Unaí	MG	16º02'57" S, 46º30'11" W	940	Soybean-fallow
20	Buritis	MG	15º17'08" S, 46º42'54" W	1008	Soybean-bean
21	Buritis	MG	15º15'59" S, 46º42'20" W	998	Soybean-fallow
22	Buritis	MG	15º49'56" S, 46º23'50" W	950	Soybean-corn
23	Buritis	MG	15º50'30" S, 46º25'21" W	950	Soybean-corn
24	Cabeceiras	GO	15º42'17" S, 47º03'27" W	910	Soybean-corn
25	Cabeceiras	GO	15º47'53" S, 47º05'32" W	920	Soybean-corn
26	Planaltina	DF	15º47'44" S, 47º24'03" W	910	Soybean-sorghum
27	Planaltina	DF	15º46'40" S, 47º25'41" W	1000	Soybean-corn
28	Catalão	GO	17º92'67" S, 47º48'87" W	840	Soybean-corn
29	Catalão	GO	17º75'66" S, 47º57'20" W	840	Soybean-corn
30	Campo Alegre de Goiás	GO	17º47'30" S, 47º79'83" W	850	Soybean-corn
31	Campo Alegre de Goiás	GO	17º44'31" S, 47º99'22" W	850	Soybean-corn
32	Catalão	GO	17º95'35" S, 47º40'66" W	850	Soybean-corn
33	Catalão	GO	18º17'44" S, 47º94'15" W	840	Soybean-corn
34	Campo Alegre de Goiás	GO	17º28'43" S, 47º83'29" W	840	Soybean-corn
35	Planaltina	GO	15º13'08" S, 47º35'12" W	1250	Soybean-fallow
36	Planaltina	GO	15º28'49" S, 47º33'12" W	960	Soybean-fallow
37	Planaltina	GO	15º24'07" S, 47º32'09" W	1150	Soybean-fallow
38	Formosa	GO	15º18'20" S, 47º29'45" W	1090	Soybean-fallow
39	Planaltina	GO	15º13'07" S, 47º31'56" W	1190	Soybean-bean
40	Planaltina	GO	15º17'57" S, 47º31'59" W	1210	Soybean-bean
41	Montividiu	GO	17º00'21" S, 51º08'01" W	830	Soybean-corn
42	Rio Verde	GO	17º38'15" S, 51º08'20" W	880	Soybean-corn
43	Rio Verde	GO	17º48'54" S, 50º53'25" W	730	Soybean-corn

Continuation...

Table 1. Continuation...

Pop.	Municipality	State(1)	Geographic coordinates	Altitude (m)	Crop (summer - off-season)
44	Jataí	GO	18º00'38" S, 52º07'39" W	839	Soybean-corn
45	Jataí	GO	18º09'91" S, 51º52'58" W	847	Soybean-corn
46	Jataí	GO	18º10'31" S, 52º02'31" W	783	Soybean-corn
47	Jataí	GO	17º38'62" S, 51º40'79" W	904	Soybean-corn
48	Jataí	GO	17º54'94" S, 51º46'06" W	707	Soybean-corn
49	Paraúna	GO	16º53'55" S, 50º26'48" W	780	Soybean-millet
50	Tuverlândia	GO	17º51'07" S, 50º17'28" W	620	Soybean-sorghum
51	Rio Verde	GO	18º02'46" S, 51º11'02" W	720	Soybean-corn
52	Rio Verde	GO	17º49'14" S, 51º03'28" W	760	Soybean-sorghum
53	Rio Verde	GO	18º08'58" S, 50º52'21" W	805	Soybean-millet
54	Indiara	GO	17º06'22" S, 49º54'03" W	570	Soybean-corn
55	Jandaia	GO	17º04'00" S, 50º60'10" W	637	Soybean-sorghum
56	Acreúna	GO	17º17'46" S, 50º23'11" W	640	Soybean-corn
57	Rio Verde	GO	17º46'27" S, 50º54'16" W	760	Soybean-corn
58	Rio Verde	GO	17º42'20" S, 50º58'19" W	770	Soybean-sorghum
59	Rio Verde	GO	17º46'18" S, 50º53'49" W	730	Soybean-corn
60	Montividiu	GO	17º47'37" S, 50º43'40" W	700	Soybean-sorghum
61	Santa Helena de Goiás	GO	17º28'14" S, 51º08'53" W	890	Soybean-corn
62	Jataí	GO	18º02'80" S, 52º01'35" W	877	Soybean-corn
63	Jataí	GO	17º53'78" S, 52º01'76" W	842	Soybean-corn
64	Jataí	GO	18º11'03" S, 51º53'05" W	814	Soybean-corn
65	Unaí	MG	16º43'15" S, 48º43'54" W	547	Soybean-fallow
66	Cristalina	GO	16º20'34" S, 47º25'19" W	859	Soybean-fallow
67	Paracatu	MG	16º39'44" S, 47º02'33" W	898	Soybean-fallow
68	Unaí	MG	16º25'09" S, 47º18'89" W	987	Soybean-bean
69	Unaí	MG	16º29'08" S, 47º29'08" W	847	Soybean-corn
70	Paracatu	MG	16º54'48" S, 47º06'31" W	892	Coffee
71	Unaí	MG	16º27'93" S, 47º07'69" W	978	Soybean-sorghum
72	Unaí	MG	16º34'43" S, 47º15'34" W	934	Soybean-fallow
73	Cabeceira Grande	MG	15º59'17" S, 47º02'79" W	949	Soybean-corn
74	Unaí	MG	16º07'89" S, 46º36'01" W	950	Soybean-corn
75	Cabeceira Grande	MG	15º05'08" S, 47º04'87" W	870	Soybean-fallow
76	Unaí	MG	15º57'79" S, 46º39'78" W	955	Soybean-sorghum
77	Unaí	MG	16º24'42" S, 47º13'94" W	981	Soybean-fallow
78	Garapuava	MG	16º08'38" S, 46º37'02" W	962	Soybean-fallow
79	Buriti Alegre	GO	18º07'63" S, 49º15'14" W	625	Soybean-corn
80	Bom Jesus de Goiás	GO	18º11'87" S, 49º47'68" W	673	Soybean-corn
81	Porteirão	GO	18º00'83" S, 50º07'16" W	507	Soybean-millet
82	Goiatuba	GO	18º00'06" S, 49º36'83" W	723	Soybean-corn
83	Edeia	GO	17º22'10" S, 49º54'61" W	690	Soybean-millet
84	Itumbiara	GO	18º12'36" S, 49º16'16" W	562	Soybean-corn
85	Goiatuba	GO	18º04'25" S, 49º17'58" W	653	Soybean-sorghum
86	Joviânia	GO	17º48'12" S, 49º39'41" W	834	Soybean-corn

Continuation...

Table 1. Continuation...

Pop.	Municipality	State(1)	Geographic coordinates	Altitude (m)	Crop (summer - off-season)
87	Cristalina	GO	16º03'32" S, 47º30'10" W	1020	Soybean-fallow
88	Cristalina	GO	16º38'44" S, 47º34'14" W	1000	Soybean-sorghum
89	Cristalina	GO	16º52'30" S, 47º22'40" W	915	Soybean-corn
90	Cristalina	GO	16º19'06" S, 47º57'01" W	906	Soybean-bean
91	Cristalina	GO	16º49'53" S, 47º44'35" W	1070	Soybean-corn
92	Patos de Minas	MG	18º37'54" S, 46º34'59" W	870	Soybean-fallow
93	Patos de Minas	MG	18º36'43" S, 46º32'26" W	830	Soybean-fallow
94	Presidente Olegário	MG	18º05'44" S, 46º27'52" W	980	Soybean-fallow
95	Patrocínio	MG	18º56'36" S, 47º02'51" W	880	Soybean-corn
96	Pedrinópolis	MG	19º11'46" S, 47º34'21" W	974	Soybean-wheat
97	Pedrinópolis	MG	19º11'10" S, 47º31'10" W	997	Soybean-fallow
98	Santa Juliana	MG	19º30'20" S, 47º25'04" W	976	Soybean-wheat
99	Unaí	MG	16º11'51" S, 46º34'35" W	940	Soybean-corn
100	Unaí	MG	16º10'13" S, 46º37'20" W	950	Soybean-corn
101	Unaí	MG	16º16'36" S, 46º25'20" W	910	Soybean-corn
102	Unaí	MG	16º18'32" S, 46º28'34" W	930	Soybean-millet
103	Unaí	MG	16º23'52" S, 47º14'07" W	970	Soybean-millet
104	Conquista	MG	19º45'36" S, 47º37'01" W	850	Soybean-fallow
105	Uberaba	MG	19º43'50" S, 47º37'46" W	825	Soybean-fallow
106	Frutal	MG	20º06'09" S, 48º55'23" W	520	Soybean-corn
107	Uberlândia	MG	19º05'07" S, 48º12'48" W	930	Soybean-fallow
108	Uberlândia	MG	19º12'44" S, 47º57'00" W	910	Soybean-fallow
109	Iraí de Minas	MG	18º58'02" S, 47º26'45" W	900	Soybean-fallow
110	Abadia dos Dourados	MG	18º27'33" S, 47º22'13" W	850	Soybean-sorghum
111	Araguari	MG	19º33'15" S, 48º20'29" W	900	Soybean-fallow
112	Tupaciguara	MG	18º40'41" S, 48º45'55" W	779	Soybean-fallow
113	Tupaciguara	MG	18º32'05" S, 48º42'25" W	913	Soybean-fallow
114	Silvânia	GO	16º42'20" S, 48º36'41" W	1018	Soybean-fallow
115	Palmeiras de Goiás	GO	16º55'06" S, 49º50'60" W	624	Soybean-fallow
116	Palmeiras de Goiás	GO	16º51'06" S, 49º51'29" W	610	Soybean-fallow
117	Morrinhos	GO	17º43'47" S, 49º01'28" W	861	Soybean-corn
118	Orizona	GO	17º03'32" S, 48º24'10" W	970	Soybean-fallow
119	Campinorte	GO	14º12'09" S, 49º08'50" W	530	Soybean-corn
120	Uruaçu	GO	14º45'46" S, 49º15'50" W	620	Soybean-corn
121	Hidrolina	GO	14º38'58" S, 49º11'09" W	580	Soybean-sorghum
122	Hidrolina	GO	14º47'52" S, 49º17'04" W	550	Soybean-millet
123	Uruaçu	GO	14º27'16" S, 49º09'24" W	770	Soybean-millet
124	Padre Bernardo	GO	15º12'34" S, 48º25'03" W	730	Soybean-fallow
125	Padre Bernardo	GO	15º01'22" S, 48º45'54" W	729	Soybean-millet
126	Padre Bernardo	GO	15º24'66" S, 48º14'93" W	736	Soybean-fallow
127	Padre Bernardo	GO	15º28'04" S, 48º14'65" W	751	Soybean-fallow
128	Padre Bernardo	GO	15º26'21" S, 48º14'89" W	763	Soybean-fallow
129	Buritis	MG	15º38'10" S, 46º27'58" W	952	Soybean-bean

Continuation...

Table 1. Continuation...

Pop.	Municipality	State[(1)]	Geographic coordinates	Altitude (m)	Crop (summer - off-season)
130	Formoso	MG	14º49'45" S, 46º29'31" W	987	Soybean-fallow
131	Formoso	MG	14º49'07" S, 46º20'99" W	908	Soybean-fallow
132	Formoso	MG	14º48'97" S, 46º20'67" W	917	Soybean-fallow
133	Buritis	MG	15º24'16" S, 46º34'12" W	940	Soybean-fallow
134	Buritis	MG	15º25'46" S, 46º27'02" W	920	Soybean-fallow
135	Padre Bernardo	GO	15º15'34" S, 48º15'44" W	720	Soybean-fallow
136	Padre Bernardo	GO	15º11'24" S, 48º32'00" W	590	Soybean-fallow
137	Santo Antônio do Descoberto	GO	16º04'31" S, 48º19'09" W	1067	Soybean-corn
138	Cristalina	GO	16º33'37" S, 47º37'00" W	1011	Coffee
139	Cristalina	GO	16º27'54" S, 47º33'35" W	920	Soybean-fallow
140	Cristalina	GO	16º47'25" S, 47º37'59" W	1190	Soybean-fallow
141	Unaí	MG	16º29'41" S, 47º24'53" W	940	Soybean-fallow
142	Cristalina	GO	16º12'05" S, 47º37'48" W	999	Soybean-cotton
143	Cristalina	GO	16º14'23" S, 47º38'06" W	983	Soybean-cotton
144	Cristalina	GO	16º24'31" S, 47º37'40" W	951	Soybean-tomato
145	Cristalina	GO	16º26'37" S, 47º37'36" W	949	Soybean-tomato
146	Cristalina	GO	16º23'34" S, 47º36'42" W	951	Soybean-fallow
147	Cristalina	GO	16º27'28" S, 47º33'51" W	928	Soybean-fallow
148	Cristalina	GO	16º27'08" S, 47º34'02" W	948	Soybean-fallow
149	Cristalina	GO	16º25'19" S, 47º33'41" W	913	Soybean-millet
150	Cristalina	GO	16º24'42" S, 47º33'21" W	886	Soybean-tomato
151	Cristalina	GO	16º25'59" S, 47º35'39" W	881	Soybean-sweet corn
152	Cristalina	GO	16º17'48" S, 47º37'38" W	975	Soybean-fallow
153	Cristalina	GO	16º03'07" S, 47º24'29" W	927	Soybean-fallow
154	Planaltina	DF	16º01'02" S, 47º26'11" W	954	Soybean-fallow
155	Planaltina	DF	15º51'29" S, 47º23'14" W	882	Soybean-wheat
156	Planaltina	DF	15º48'10" S, 47º38'16" W	1034	Soybean-fallow
157	Planaltina	DF	15º43'29" S, 47º23'21" W	991	Soybean-corn
158	Planaltina	DF	15º53'15" S, 47º23'29" W	890	Soybean-bean
159	Planaltina	DF	15º57'48" S, 47º35'24" W	1020	Soybean-millet
160	Planaltina	DF	16º00'29" S, 47º29'46" W	998	Soybean-fallow
161	Uberlândia	MG	18º53'46" S, 48º17'28" W	865	Standard population

(1)Brazilian federative units: MG, state of Minas Gerais; GO, state of Goiás; and DF, Distrito Federal.

tests were carried out in a greenhouse (15º56'02.10"S, 48º08'15.94"W, at 993 m altitude), under controlled temperature at 30ºC (day) and 20ºC (night), and 12-hour photoperiod, from April 2017 to May 2018, using a completely randomized design, with three replicates.

Sixteen sensitivity tests were carried out, according to the receipt of seed samples collected in the field. A standard population (susceptible population to the herbicides) was maintained and used in each test to grade the weed control for comparison. The tested herbicides were the following ones: glyphosate, at 1,000 g ha^{-1} of acid equivalent (a.e.); clethodim, at 108 g ha^{-1} of the active ingredient (a.i.); and haloxyfop-P-methyl at 62.35 g ha^{-1} a.i. A control treatment with no herbicide application was used for each population. The herbicide rates were established based on the recommendation of the products for *D. insularis* (Rodrigues & Almeida, 2018).

D. insularis seed were sown in expanded polystyrene trays for seedling formation. The seedlings were transplanted to pots at 15 days after sowing, and then thinned to maintain two plants only per pot. The experimental unit consisted of 2.0 dm^3 plastic pots, with a substrate of soil, sand, and plant compost mixture (3:1:1), fertilized with N, P, and K, at 100, 200, and 150 mg kg^{-1}, respectively. Each pot was placed on a plastic container of large diameter, and without holes to maintain the water regime of the plots. Soil moisture was controlled daily, with water replenishing in the containers when necessary.

The herbicides were applied when the plants had 3 to 4 tillers using a CO_2 pressurized backpack sprayer with constant-pressure of 2.8 kgf cm^{-2}. The sprayer was equipped with a bar with two flat jet tips (TTI110015) spaced 0.5 m apart. The application volume was equivalent to 200 L ha^{-1}.

Visual evaluations of control were carried out at 15 and 30 days after the herbicide application (DAA), using a 0 to 100% scale of grades, in which zero represents the absence of visual injuries, and 100 represents plant death (Velini et al., 1995). The grades were attributed to each plant of the plot (pot), and the mean control per replicate (pot) was calculated. The mean control was used for the statistical analysis.

Supposedly resistant populations were compared with the susceptible ones and classified as susceptible (>80% control, without regrowth), intermediately resistant (population with susceptible and resistant plants), or resistant (0 to 79% of control, with regrowth) to the herbicides. Population was classified as intermediate when one resistant plant was found in a plot, even if the other plants in the plot were susceptible. Intermediate resistance is indicative of the existence of resistant individuals in the evaluated population, but not with all plants showing resistance.

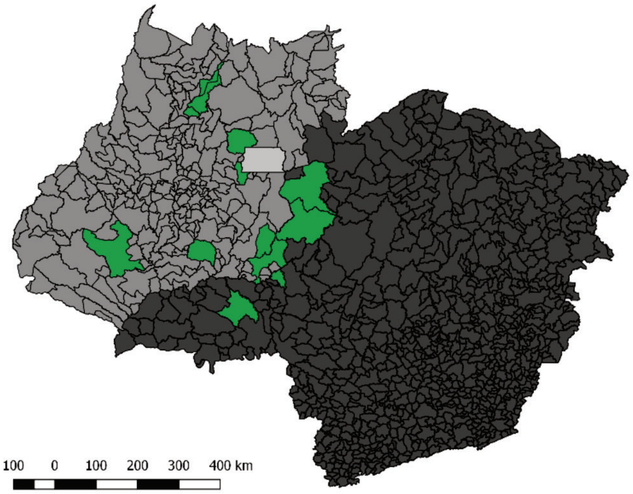
The results were used to develop maps of infestation, with the distribution of cases of resistance by species and herbicide in the evaluated municipalities, using the QGIS 2.18 program (Graser & Peterson, 2016). The maps of the evaluated states [shape files (SHP); 2017 version] were obtained from the website of the Brazilian Institute of Geography and Statistics (IBGE, 2018).

The grades of control (mean values per plot) were subjected to analysis of variance, in order to assess the response variability of weed populations to the tested herbicides. After that procedure, dispersion graphs were developed and the different susceptibility responses to the herbicides were analyzed by hierarchical clustering through the unweighted pair group method with arithmetic mean (UPGMA), using the Toucher method to separate the groups (Silva, 2016).

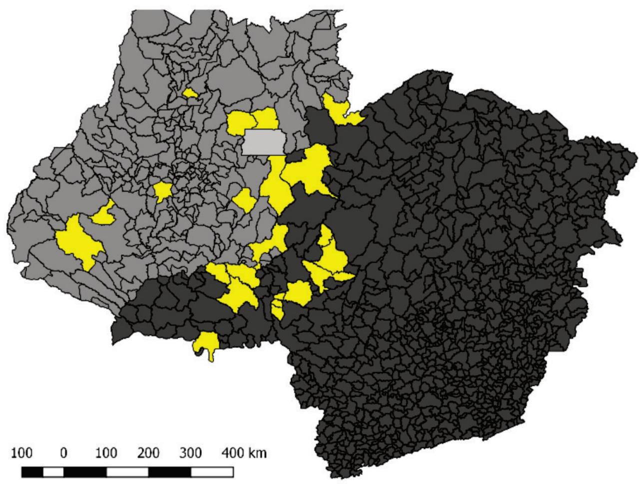
Results and discussion

Considering the classification of populations as susceptible, intermediately resistant, and resistant, all weed populations were susceptible to clethodim; 97.5% were susceptible (Figure 1) and 2.5% intermediately resistant to haloxyfop-P-methyl (Figure 2); and 9.9% were susceptible, 21.1% intermediately resistant, and 68.9% resistant to glyphosate (Figure 3). The results confirmed the dispersion of glyphosate-resistant *D. insularis* plants in the Cerrado biome, in Brazil. According to Takano et al. (2018), glyphosate-resistant *D. insularis* populations can evolve through independent selections, contributing to an expressive dissemination of resistance in agricultural areas. However, despite the survival of *D. insularis* plants in the field, after glyphosate applications, most farmers continue to use only glyphosate for weed management, since effective management strategies require associations of herbicides (Zobiole et al., 2016), which increases the production costs (Adegas et al., 2018).

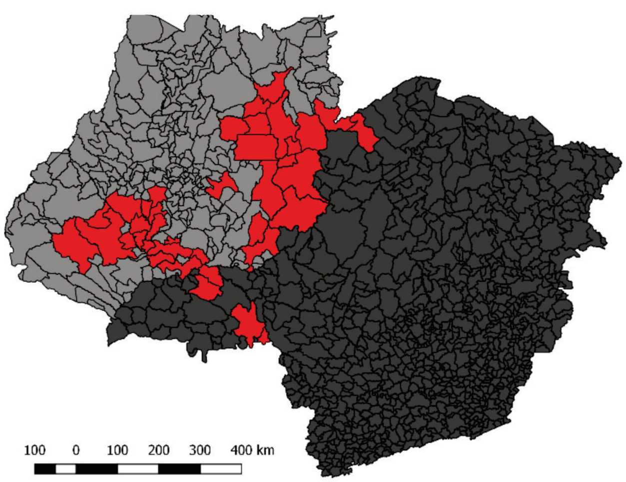
Populations with intermediate resistance to haloxyfop-P-methyl were found in the municipalities of Abadia dos Dourados (Minas Gerais state), and Montividiu, Padre Bernardo, and Rio Verde (Goiás state). These results raised attention to some areas that were subjected to applications of haloxyfop-P-methyl, since control problems for these populations have been found. The main strategy adopted in agricultural areas for the management of adult *D. insularis* plants resistant to glyphosate is the use of ACCase-inhibitor herbicides, mainly clethodim and haloxyfop-P-methyl, which are applied at high rates, often above those recommended by the manufacturers, and with sequential applications (Zobiole et al., 2016). This is certainly a problem, since it increases the selection pressure for multiple resistance to glyphosate and ACCase inhibitors, especially haloxyfop-P-methyl,



Susceptible
Abadia dos Dourados, MG (1)
Campinorte, GO (1)
Campo Alegre de Goiás, GO (1)
Catalão, GO (1)
Hidrolina, GO (1)
Morrinhos, GO (1)
Padre Bernardo, GO (1)
Paracatu, MG (1)
Rio Verde, GO (2)
Santo Antônio do Descoberto, GO (1)
Uberlândia , MG (1)
Unaí, MG (1)
Uruaçu, GO (2)



Intermediately
Araguari, MG (1)
Catalão, GO (1)
Cristalina, GO (4)
Formoso, MG (2)
Frutal, MG (1)
Hidrolina, GO (1)
Iraí de Minas, MG (1)
Jataí, GO (2)
Lagoa Grande, MG (1)
Montividiu, GO (1)
Orizona, GO (1)
Padre Bernardo, GO (1)
Palmeiras de Goias, GO (1)
Patos de Minas, MG (2)
Pedrinópolis, MG (2)
Planaltina, GO (1)
Patrocínio, MG (1)
Presidente Olegário, MG (1)
Santa Juliana, MG (1)
Tupaciguara, MG (2)
Uberlândia, MG (4)
Unaí, MG (2)



Resistant
Acreúna, GO (2)
Água Fria de Goiás, GO (1)
Bom Jesus de Goiás, GO (1)
Buriti Alegre, GO (1)
Buritis, MG (12)
Cabeceira Grande, MG (2)
Cabeceiras, GO (2)
Campo Alegre de Goiás, GO (2)
Catalão, GO (2)
Chapada Gaúcha, MG (1)
Conquista, MG (1)
Cristalina, GO (17)
Edeia, GO (1)
Formosa, GO (1)
Formoso, MG (1)
Goiatuba, GO (2)
Indiara, GO (1)
Itumbiara, GO (1)
Jandaia, GO (1)
Jataí, GO (6)
Joviânia, GO (1)
Monte Alegre de Minas, MG (1)
Montividiu, GO (1)
Padre Bernardo, GO (9)
Palmeiras de Goias, GO (1)
Paracatu, MG (2)
Paraúna, GO (1)
Planaltina, DF (9)
Planaltina, GO (4)
Porteirão, GO (1)
Rio Verde, GO (6)
Santa Helena de Goiás, GO (1)
São João da Aliança, GO (1)
Silvânia, GO (1)
Tupaciguara, MG (2)
Tuverlândia, GO (1)
Uberaba, MG (1)
Unaí, MG (10)

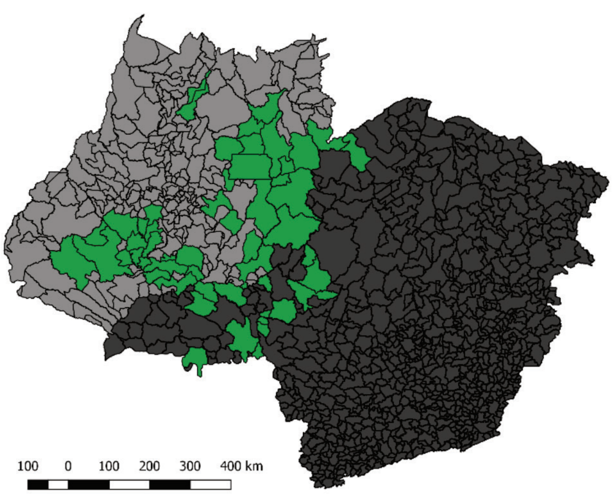
Figure 1. Response of *Digitaria insularis* populations to glyphosate: 9.9% susceptible (green); 21.1% intermediately resistant (yellow); and 68.9% resistant (red).

as it have occurred for *Eleusine indica* in Brazil in 2017 (Heap, 2020). Multiple resistance is the ability of plants to survive to applications of herbicides with two or more mechanisms of action (Christoffoleti & López Ovejero, 2008).

Resistant *D. insularis* to ACCase-inhibitor herbicides in Brazil was caused by the mutation Trp2027Cys in the action site, which does not allow of the connection of several herbicides of the aryloxyphenoxypropionate group to it, such as haloxyfop-P-methyl and fenoxaprop-P-ethyl (Takano et al., 2020). However, all populations were susceptible to clethodim, confirming the importance of this herbicide for the management of *D. insularis*. Moreover, good agricultural practices should be used to avoid the evolution of new cases of resistance and multiple resistance, including the use of soil cover plants, crop rotation, mixture of herbicides, and the association of chemical and mechanical weed control methods (Marochi et al., 2018; Raimondi et al., 2020).

Populations with resistance to glyphosate were found in 38 of the 56 evaluated municipalities, and 10 municipalities showed populations with intermediate resistance, indicating the presence of resistant plants, but at a lower frequency. Thus, more than 85% of the municipalities displayed already resistant biotypes in at least one of their agricultural areas. These resistant populations were homogeneously distributed in the evaluated regions and not concentrated in a region, or state. Susceptible populations to glyphosate were found in 11 municipalities in the states of Goiás and Minas Gerais. Not all plants that survive to glyphosate applications in the field are resistant to the herbicide. This is explained by the soil moisture conditions and plant size at the time of application. Glyphosate is mainly applied to control adult *D. insularis* plants that show flowers, fruit, and seed (Zobiole et al., 2016; Raimondi et al., 2020), which requires good soil moisture conditions for an adequate absorption, translocation, and action of glyphosate on the plants. Therefore, sometimes the herbicide is not effective due to water stress of plants at the time of application.

According to the analysis of variance, the percentage of control of the *D. insularis* populations was significantly different (p<0.01), both at 15 and 30 DAA, indicating a variability in their response to



Susceptible
Abadia dos Dourados, MG (1)
Acreúna, GO (1)
Água Fria de Goiás, GO (1)
Araguari, MG (1)
Bom Jesus de Goiás, GO (1)
Buriti Alegre, GO (1)
Buritis, MG (12)
Cabeceira Grande, MG (2)
Cabeceiras, GO (2)
Campinorte, GO (1)
Campo Alegre de Goiás, GO (3)
Catalão, GO (4)
Chapada Gaúcha, MG (1)
Conquista, MG (1)
Cristalina, GO (21)
Edeia, GO (1)
Formosa, GO (1)
Formoso, MG (3)
Frutal, MG (1)
Guarapuava, MG (1)
Goiatuba, GO (2)
Hidrolina, GO (2)
Indiara, GO (1)
Iraí de Minas, MG (1)
Itumbiara, GO (1)
Jandaia, GO (1)
Jataí, GO (8)
Lagoa Grande, MG (1)
Monte Alegre de Minas, MG (1)
Montividiu, GO (2)
Morrinhos, GO (1)
Orizona, GO (1)
Padre Bernardo, GO (11)
Palmeiras de Goiás, GO (2)
Paracatu, MG (2)
Paraúna, GO (1)
Patos de Minas, MG (2)
Pedrinópolis, MG (2)
Planaltina, DF (9)
Planaltina, GO (5)
Porteirão, GO (1)
Patrocínio, MG (1)
Presidente Olegário, MG (1)
Rio Verde, GO (8)
Santa Helena de Goiás, GO (1)
Santa Juliana, MG (1)
Santo Antônio do Descoberto, GO(1)
São João da Aliança, GO (1)
Silvânia, GO (1)
Tupaciguara, MG (4)
Tuverlândia, GO (1)
Uberaba, MG (1)
Unaí, MG (15)
Uruaçu, GO (2)

Figure 2. Response of *Digitaria insularis* populations to clethodim: 100% susceptible (green).

the tested herbicides. Different susceptibility of weed populations to the herbicides was also found through the hierarchical cluster analysis by the Toucher method, which allowed of the formation of 22, 9, and 2 groups of susceptibility to glyphosate, clethodim, and haloxyfop-P-methyl, respectively (Table 2).

The *D. insularis* populations were, in general, not effectively controlled by the glyphosate herbicide, except for 12 populations that showed ≥80% control at 30 DAA (Figure 4 A), and fitted to the groups I to V, according to the Toucher's method. The other populations were unsatisfactorily controlled (<80%) or not controlled (0%), characterizing them as intermediately resistant populations or resistant populations to glyphosate. These populations grouped into 22 groups, most of them (87.6%) concentrated in the groups VI to XXII, with controls below 80%. The populations in these groups of lower sensitivity showed a high amplitude of response to the herbicide (0 to 79.2%).

Eleven populations were the most susceptible ones to the herbicide (Group I), with control grades from 99% to 100%. These populations were from agricultural areas in the municipalities of Abadia dos Dourados and Unaí, in the state of Minas Gerais; and Campinorte, Hidrolina, Morrinhos, Padre Bernardo, Rio Verde, Santo Antônio do Descoberto, and Uruaçu, in the state of Goiás, Brazil. None of these areas was kept with weeds, with seed production and increase of the soil seed bank between crop seasons. All these areas were cultivated with soybean, in the spring-summer season, combined with other crops in

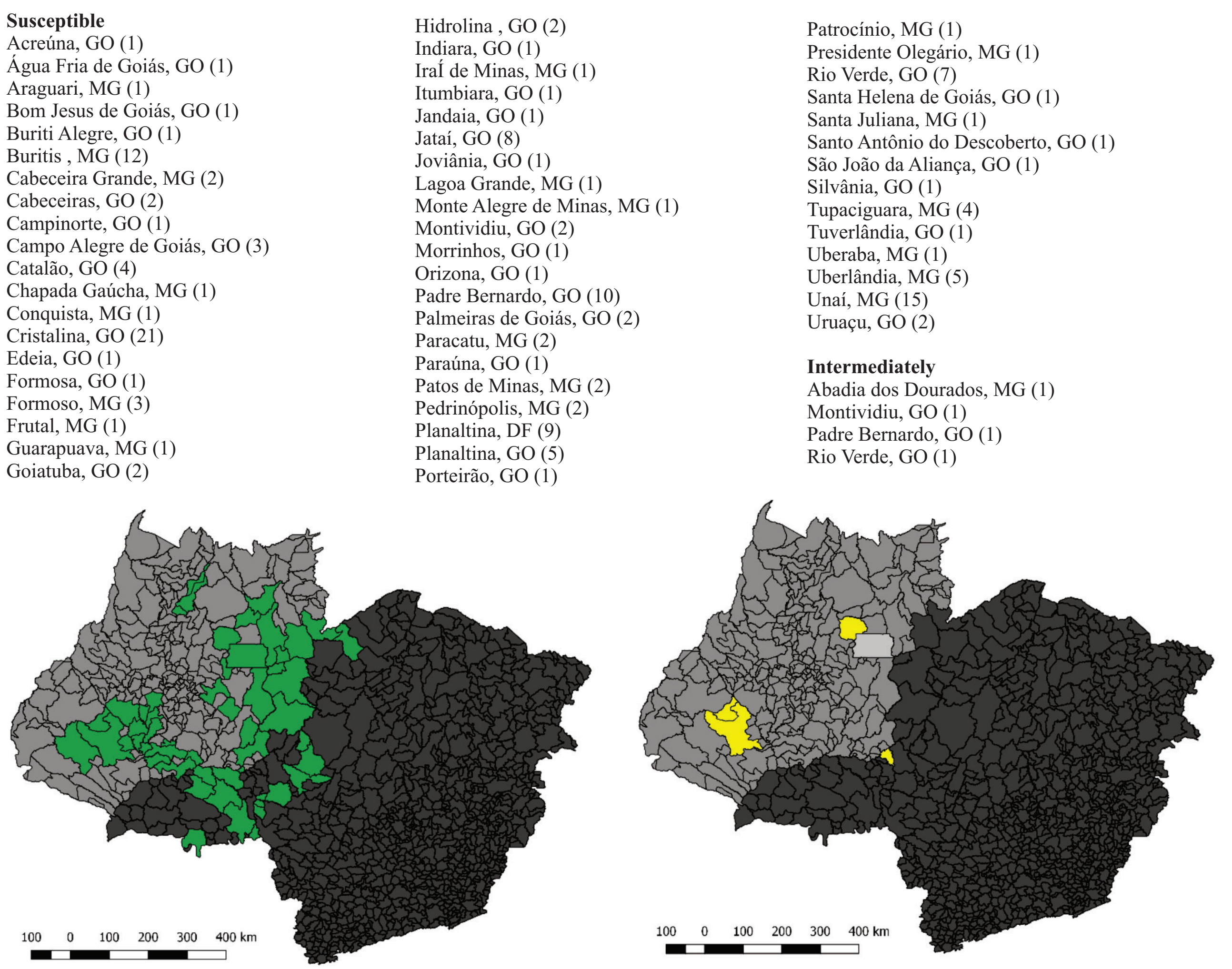


Figure 3. Sensitivity of *Digitaria insularis* populations to haloxyfop-P-methyl: 97.5% susceptible (green) and 2.5 intermediately resistant (yellow).

Table 2. *Digitaria insularis* grouping of 16 populations as a function of susceptibility to the herbicides glyphosate (1,000 g ha^{-1} a.e.), clethodim (108 g ha^{-1} a.i.), and haloxyfop-P-methyl (62.35 g ha^{-1} a.i.), using the Toucher's method.

Herbicide	Group	Control interval (%)	Population[(1)]
Glyphosate	I	99.0–100.0	101, 103, 110, 117, 119, 120, 122, 123, 137, 125, 52
	II	92.5–95.0	41, 69, 58
	III	89.2	32
	IV	85.0	4
	V	80.0–82.5	106, 113, 1
	VI	75.0–79.2	2, 94, 97, 99, 108, 132, 95, 98, 109, 116
	VII	6.7–71.7	5, 145, 105, 45, 89, 128, 104, 90, 102, 121
	VIII	61.7–65.0	88, 92, 44, 31, 96
	XIX	58.3–60.0	111, 140
	X	53.3–56.7	86, 131, 100, 93, 142, 114
	XI	50.0	133, 134, 149
	XII	45.0–45.8	107, 115, 63
	XIII	38.3–43.3	7, 15, 28, 39, 67, 126, 127, 150, 3, 156, 157, 18, 87, 144
	XIV	33.3–36.7	112, 155, 135, 17
	XV	30.0	14
	XVI	21.7–26.7	8, 9, 36, 26, 154, 59, 118
	XVII	20.0	22, 24, 56, 76, 138, 153, 158
	XVIII	16.7	10, 143
	XIX	13.3	16, 66, 84, 91, 130, 146
	XX	8.3–10.0	29, 37, 49, 57, 60, 129, 136, 139, 147, 148, 43
	XXI	6.7	12, 46, 54, 77, 81, 83, 33, 13, 42, 50, 51, 55, 65
	XXII	0.0	6, 11, 19, 20, 21, 23, 25, 27, 30, 34, 35, 38, 40, 47, 48, 53, 61, 62, 64, 68, 70, 71, 72, 73, 74, 75, 78, 79, 80, 82, 85, 124, 141, 151, 152, 159, 160
Clethodim	I	99.2–100.0	1, 4, 8, 12, 14, 15, 19, 22, 24, 27, 28, 29, 32, 34, 35, 37, 38, 39, 40, 42, 53, 56, 63, 69, 70, 73, 74, 75, 80, 87, 88, 93, 94, 95, 96, 98, 99, 100, 102, 107, 111, 112, 113, 115, 116, 117, 118, 119, 120, 122, 123, 136, 137, 139, 140, 141, 153, 157, 61, 89, 90, 91, 92, 103, 106, 121, 128, 129, 135, 161, 3, 5, 101, 110, 114, 125, 132, 9, 23, 31, 36, 43, 46, 62, 64, 67, 72, 108, 126, 134, 156
	II	98.8–99.0	17, 131, 133, 109, 149
	III	97.5–98.5	6, 11, 18, 25, 30, 41, 47, 49, 51, 55, 65, 68, 86, 124, 10, 58, 105, 130, 145, 7, 16, 33, 44, 50, 71, 76, 77, 82, 85, 97, 138, 104
	IV	96.7	21, 26, 54, 60, 83, 84, 147, 155, 48
	V	95.8	45, 57, 59, 66
	VI	94.2–95.0	13, 79, 81, 158, 142, 52, 78, 127
	VII	92.5–93.3	2, 148, 160, 20, 151, 159
	VIII	91.7	143, 146, 150
	IX	90.0–90.8	144, 152, 154
Haloxyfop-P-methyl	I	89.6–100.0	1, 8, 75, 106, 29, 74, 80, 141, 5, 99, 61, 108, 42, 56, 69, 94, 87, 114, 67, 105, 22, 14, 15, 62, 73, 17, 70, 82, 4, 109, 135, 93, 64, 104, 9, 122, 130, 139, 140, 123, 48, 11, 112, 16, 43, 47, 53, 23, 91, 24, 50, 101, 71, 97, 55, 72, 83, 7, 51, 49, 77, 129, 10, 155, 90, 85, 3, 25, 98, 119, 6, 124, 57, 59, 68, 13, 46, 44, 76, 102, 113, 120, 118, 54, 88, 153, 103, 95, 96, 84, 131, 65, 63, 132, 137, 18, 66, 31, 60, 89, 128, 26, 117, 92, 121, 37, 126, 12, 100, 111, 19, 81, 161, 20, 27, 79, 134, 32, 110, 127, 115, 116, 138, 145, 78, 86, 21, 38, 45, 133, 28, 107, 2, 136, 58, 150, 147, 149, 125, 33, 158, 34 ,157, 30, 36, 156, 159, 143, 160, 152, 151, 144, 40, 39, 35, 146, 154, 148, 142
	II	16.2–33.3	41, 52

[(1)]Populations were organized from the greater control to the lower one, with basis on the group's control interval.

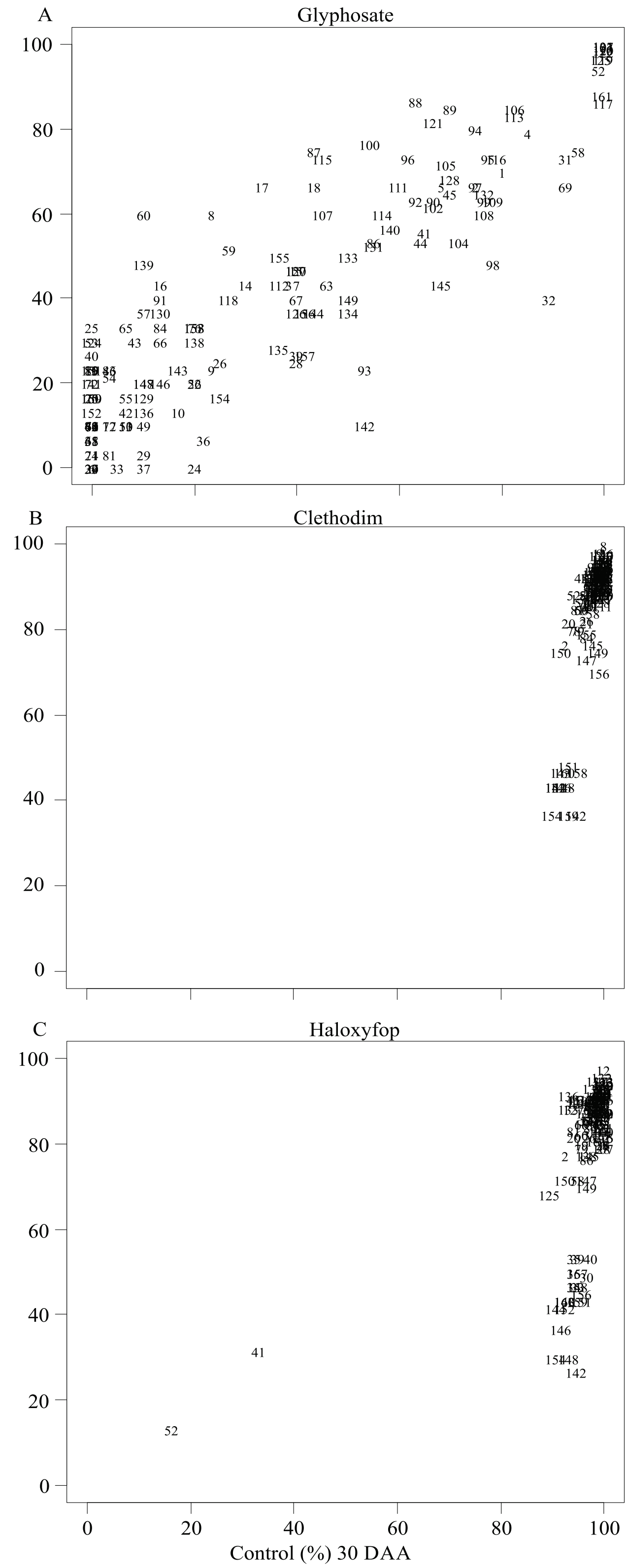


Figure 4. Dispersion of the grade control of *Digitaria insularis* populations, at 15 and 30 days after application (DAA) of glyphosate (1,000 g ha^{-1} a.e.), clethodim (108 g ha^{-1} a.i.), and haloxyfop-P-methyl (62.35 g ha^{-1} a.i.) herbicides.

the autumn-winter season, such as maize, millet, and sorghum, confirming that the best strategy to prevent weed resistance to herbicides is the use of different management strategies, starting by the diversification of crops in the area (Norsworthy et al., 2012; Marochi et al., 2018).

Clethodim displayed an excellent control (≥90%) of all *D. insularis* populations at 30 DAA. Significant differences were found only at 15 DAA, with 10 populations showing lower-control percentages (Figure 4 B). The Toucher's method showed a high-discrimination power of the clethodim herbicide, forming nine groups of similar populations. However, the analysis showed a low-dissimilarity degree due to the low variation of control grades between groups. Despite the formation of several groups, the control obtained was satisfactory, and the populations were classified as susceptible.

The susceptibility of the populations to haloxyfop-P-methyl was different at 15 DAA for 34 populations, which were more tolerant (<80% of control) to this herbicide than the others (Figure 4 C). However, haloxyfop-P-methyl promoted an excellent control of *D. insularis* at 30 DAA, except for the populations 41 and 52, for which the herbicide was ineffective. This fact was also observed in the analysis by the Toucher's method, which formed two groups: a susceptible group (group I, with 89.6 to 99.7% control) constituted by 98.8% of the total evaluated populations; and a tolerant group (group II) with the populations 41 (from Montividiu, with 33.3% control) and 52 (from Rio Verde, with 16.3% control).

The map of susceptibility to haloxyfop-P-methyl and the statistical results showed discrepancies. Based on the classification, four populations were intermediately resistant (groups 41, 52, 110, and 125), but only two of them were confirmed by the statistical analysis. This can be explained by the mean of control in the statistics, which was calculated using individual grades of the plants of each replicate. Thus, the control means of the populations 110 and 125, which had only one (110) or two (125) resistant plants, were not significant in comparison to the control means of populations that had four to five resistant plants among the six evaluated ones.

Populations 41, 52, 110, and 125, that had control problems because of haloxyfop-P-methyl applications, showed resistant biotypes; they were susceptible to glyphosate and grouped in group I (populations 52, 110, and 125) and group II (population 41), with an excellent control. Therefore, these populations are not related to possible cases of multiple resistance.

Conclusions

1. The high percentage of resistant *Digitaria insularis* populations to the herbicide glyphosate are homogeneously distributed throughout the evaluated regions in the Brazilian Cerrado biome.

2. The sampled regions shows no cases of multiple resistance of *D. insularis* to EPSPs and acetyl-CoA carboxylase (ACCase) inhibitor herbicides, but displays a cross-resistance to glyphosate and haloxyfop-P-methyl.

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