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

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, intermediate 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 intermediate resistant to haloxyfop-P-methyl; and 9.9% were susceptible, 21.1% intermediate 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 complaints 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 | Geographic coordinates     | Altitude (m) | Crop (summer - off-season) |
|------|-------------------|-------|----------------------------|-------------|---------------------------|
| 1    | Lagoa Grande      | MG    | 17°76'05" S, 46°53'37" W   | 557         | Soybean-fallow            |
| 2    | Monte Alegre      | MG    | 19°00'85" S, 48°58'77" W   | 702         | Soybean-fallow            |
| 3    | Uberlândia        | MG    | 18°51'42" S, 48°26'40" W   | 828         | Soybean-fallow            |
| 4    | Uberlândia        | MG    | 18°50'89" S, 48°25'65" W   | 833         | Soybean-fallow            |
| 5    | Padre Bernardo    | GO    | 15°19'00" S, 48°22'30" W   | 700         | Soybean-fallow            |
| 6    | Tupaciguara       | MG    | 18°35'04" S, 48°51'39" W   | 877         | Soybean-fallow            |
| 7    | Tupaciguara       | MG    | 18°35'66" S, 48°53'01" W   | 820         | Soybean-fallow            |
| 8    | Buritis           | MG    | 15°43'77" S, 46°24'98" W   | 919         | Soybean-fallow            |
| 9    | Buritis           | MG    | 15°43'26" S, 46°25'11" W   | 920         | Soybean-corn              |
| 10   | Buritis           | MG    | 15°29'79" S, 46°29'79" W   | 922         | Soybean-corn              |
| 11   | Buritis           | MG    | 15°48'03" S, 46°25'51" W   | 914         | Soybean-corn              |
| 12   | Buritis           | MG    | 15°42'39" S, 46°21'32" W   | 907         | Soybean-corn              |
| 13   | Chapada Gaucha    | GO    | 15°39'05" S, 45°35'47" W   | 835         | Soybean-fallow            |
| 14   | Água Fria de Goiás| GO    | 14°48'94" S, 47°39'92" W   | 1198        | Soybean-fallow            |
| 15   | São João da Aliança| GO | 14°42'60" S, 47°35'50" W | 1199        | Soybean-fallow            |
| 16   | Padre Bernardo    | GO    | 15°24'31" S, 48°26'34" W   | 668         | Soybean-fallow            |
| 17   | Padre Bernardo    | GO    | 15°21'45" S, 48°26'33" W   | 755         | Soybean-fallow            |
| 18   | Padre Bernardo    | GO    | 15°20'00" S, 48°25'13" W   | 750         | Soybean-fallow            |
| 19   | Unai              | MG    | 16°02'57" S, 46°30'11" W   | 940         | Soybean-fallow            |
| 20   | Buritis           | MG    | 15°17'08" S, 46°42'34" W   | 1008        | Soybean-bean              |
| 21   | Buritis           | MG    | 15°15'59" S, 46°42'20" W   | 998         | Soybean-fallow            |
| 22   | Buritis           | MG    | 15°49'56" S, 46°23'50" W   | 950         | Soybean-corn              |
| 23   | Buritis           | MG    | 15°50'30" S, 46°25'21" W   | 950         | Soybean-corn              |
| 24   | Cabeceiras        | GO    | 15°42'17" S, 47°03'27" W   | 910         | Soybean-corn              |
| 25   | Cabeceiras        | GO    | 15°47'53" S, 47°05'32" W   | 920         | Soybean-corn              |
| 26   | Planaltina        | DF    | 15°47'44" S, 47°24'03" W   | 910         | Soybean-sorghum           |
| 27   | Planaltina        | DF    | 15°46'40" S, 47°25'41" W   | 1000        | Soybean-corn              |
| 28   | Catalão           | GO    | 17°92'67" S, 47°48'87" W   | 840         | Soybean-corn              |
| 29   | Catalão           | GO    | 17°75'66" S, 47°57'20" W   | 840         | Soybean-corn              |
| 30   | Campo Alegre de Goiás| GO | 17°47'30" S, 47°79'83" W | 850         | Soybean-corn              |
| 31   | Campo Alegre de Goiás| GO | 17°44'31" S, 47°99'22" W | 850         | Soybean-corn              |
| 32   | Catalão           | GO    | 17°95'35" S, 47°40'66" W   | 850         | Soybean-fallow            |
| 33   | Catalão           | GO    | 18°17'44" S, 47°94'15" W   | 840         | Soybean-corn              |
| 34   | Campo Alegre de Goiás| GO | 17°28'43" S, 47°83'29" W | 840         | Soybean-corn              |
| 35   | Planaltina        | GO    | 15°13'08" S, 47°35'12" W   | 1250        | Soybean-fallow            |
| 36   | Planaltina        | GO    | 15°28'49" S, 47°33'12" W   | 960         | Soybean-fallow            |
| 37   | Planaltina        | GO    | 15°24'07" S, 47°32'09" W   | 1150        | Soybean-fallow            |
| 38   | Formosa           | GO    | 15°18'20" S, 47°29'45" W   | 1090        | Soybean-fallow            |
| 39   | Planaltina        | GO    | 15°13'07" S, 47°31'56" W   | 1150        | Soybean-bean              |
| 40   | Planaltina        | GO    | 15°17'57" S, 47°31'59" W   | 1210        | Soybean-bean              |
| 41   | Montividiu        | GO    | 17°00'21" S, 51°08'01" W   | 830         | Soybean-corn              |
| 42   | Rio Verde         | GO    | 17°38'15" S, 51°08'20" W   | 880         | Soybean-corn              |
| 43   | Rio Verde         | GO    | 17°48'54" S, 50°53'25" W   | 730         | Soybean-corn              |

Continuation...
Table 1. Continued...

| Pop. | Municipality   | State | 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   | Jandaí         | GO    | 17º06'22" S, 49º54'03" W      | 570          | Soybean-corn              |
| 55   | Jandaí         | GO    | 17º04'00" S, 50º00'10" 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    | 18º02'30" 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   | Unai           | 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   | Unai           | MG    | 16º25'09" S, 47º18'89" W      | 987          | Soybean-bean              |
| 69   | Unai           | MG    | 16º29'08" S, 47º29'08" W      | 847          | Soybean-corn              |
| 70   | Paracatu       | MG    | 16º54'48" S, 47º06'31" W      | 892          | Coffee                    |
| 71   | Unai           | MG    | 16º27'93" S, 47º07'69" W      | 978          | Soybean-sorghum           |
| 72   | Unai           | MG    | 16º34'43" S, 47º15'34" W      | 934          | Soybean-fallow            |
| 73   | Cabeceira Grande | MG | 15º59'17" S, 47º02'79" W | 949 | Soybean-corn |
| 74   | Unai           | MG    | 16º07'89" S, 46º36'01" W      | 950          | Soybean-corn              |
| 75   | Cabeceira Grande | MG | 15º05'08" S, 47º04'87" W | 870 | Soybean-fallow |
| 76   | Unai           | MG    | 15º57'79" S, 46º39'78" W      | 955          | Soybean-sorghum           |
| 77   | Unai           | 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   | Born 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   | Jovínia        | GO    | 17º48'12" S, 49º39'41" W      | 834          | Soybean-corn              |

Continuation...
Table 1. Continuation...

| Pop. | Municipality       | State | 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   | 900          | Soybean-sorghum           |
| 95   | Patrocínio        | MG    | 18º56'36" S, 47º22'40" W   | 915          | 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-sorghum           |
| 99   | Unai              | MG    | 16º11'51" S, 46º34'35" W   | 940          | Soybean-corn              |
| 100  | Unai              | MG    | 16º10'13" S, 46º37'20" W   | 950          | Soybean-fallow            |
| 101  | Unai              | MG    | 16º16'32" S, 46º28'34" W   | 930          | Soybean-millet            |
| 102  | Unai              | MG    | 16º23'52" S, 47º14'07" W   | 970          | Soybean-millet            |
| 103  | Conquista         | MG    | 19º45'36" S, 47º37'01" W   | 850          | Soybean-fallow            |
| 104  | Uberaba           | MG    | 19º43'50" S, 47º37'46" W   | 825          | Soybean-fallow            |
| 105  | Frutal            | MG    | 20º06'09" S, 48º55'23" W   | 520          | Soybean-corn              |
| 106  | Uberlândia        | MG    | 19º05'07" S, 48º12'48" W   | 930          | Soybean-fallow            |
| 107  | Iraí de Minas     | MG    | 18º58'02" S, 47º26'45" W   | 900          | Soybean-sorghum           |
| 108  | Abadia dos Dourados | MG  | 18º27'33" S, 47º21'13" W   | 850          | Soybean-corn              |
| 109  | Araguari          | MG    | 19º33'15" S, 48º20'29" W   | 900          | Soybean-fallow            |
| 110  | Tupaciguara       | MG    | 18º40'41" S, 48º45'55" W   | 779          | Soybean-fallow            |
| 111  | Palmeiras de Goiás | GO  | 16º55'06" S, 49º50'60" W   | 624          | Soybean-fallow            |
| 112  | Palmeiras de Goiás | GO  | 16º51'06" S, 49º51'29" W   | 610          | Soybean-fallow            |
| 113  | Morrinhos         | GO    | 17º43'47" S, 49º01'28" W   | 861          | Soybean-corn              |
| 114  | Orizona           | GO    | 17º03'32" S, 48º24'10" W   | 970          | Soybean-fallow            |
| 115  | Campinorte        | GO    | 14º12'09" S, 49º08'50" W   | 530          | Soybean-corn              |
| 116  | Uruaçu            | GO    | 14º45'46" S, 49º15'00" W   | 620          | Soybean-corn              |
| 117  | Hidrolina         | GO    | 14º38'58" S, 49º11'09" W   | 580          | Soybean-sorghum           |
| 118  | Buritis           | MG    | 15º38'10" S, 46º27'58" W   | 952          | Soybean-bean              |

Continuation...
tests were carried out in a greenhouse (15°56’02.10"S, 48°08’15.94"W, at 993 m altitude), under controlled temperature at 30°C (day) and 20°C (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

| Pop. | Municipality            | State | Geographic coordinates      | Altitude (m) | Crop (summer - off-season) |
|------|-------------------------|-------|-----------------------------|--------------|----------------------------|
| 130  | Formoso                 | MG    | 14°49’45” S, 46°29’31” W    | 987          | Soybean-fallow             |
| 131  | Formoso                 | MG    | 14°49’07” S, 46°20’99” W    | 908          | Soybean-fallow             |
| 132  | Formoso                 | MG    | 14°48’97” S, 46°20’67” W    | 917          | Soybean-fallow             |
| 133  | Buritis                 | MG    | 15°24’16” S, 46°34’12” W    | 940          | Soybean-fallow             |
| 134  | Buritis                 | MG    | 15°25’46” S, 46°27’02” W    | 920          | Soybean-fallow             |
| 135  | Padre Bernardo          | GO    | 15°15’34” S, 48°15’44” W    | 720          | Soybean-fallow             |
| 136  | Padre Bernardo          | GO    | 15°11’24” S, 48°32’00” W    | 590          | Soybean-fallow             |
| 137  | Santo Antônio do Descoberto | GO | 16°04’31” S, 48°19’09” W   | 1067         | Soybean-corn               |
| 138  | Cristalina              | GO    | 16°33’37” S, 47°37’00” W    | 1011         | Coffee                     |
| 139  | Cristalina              | GO    | 16°27’54” S, 47°33’35” W    | 920          | Soybean-fallow             |
| 140  | Cristalina              | GO    | 16°47’25” S, 47°37’59” W    | 1190         | Soybean-cotton             |
| 141  | Unai                    | MG    | 16°29’41” S, 47°24’53” W    | 940          | Soybean-fallow             |
| 142  | Cristalina              | GO    | 16°12’05” S, 47°34’48” W    | 999          | Soybean-cotton             |
| 143  | Cristalina              | GO    | 16°14’23” S, 47°38’06” W    | 983          | Soybean-cotton             |
| 144  | Cristalina              | GO    | 16°24’31” S, 47°37’40” W    | 951          | Soybean-tomato             |
| 145  | Cristalina              | GO    | 16°26’37” S, 47°37’36” W    | 949          | Soybean-tomato             |
| 146  | Cristalina              | GO    | 16°23’34” S, 47°36’42” W    | 951          | Soybean-fallow             |
| 147  | Cristalina              | GO    | 16°27’28” S, 47°33’51” W    | 928          | Soybean-fallow             |
| 148  | Cristalina              | GO    | 16°27’08” S, 47°34’02” W    | 948          | Soybean-fallow             |
| 149  | Cristalina              | GO    | 16°25’19” S, 47°33’41” W    | 913          | Soybean-millet             |
| 150  | Cristalina              | GO    | 16°24’42” S, 47°33’21” W    | 886          | Soybean-tomato             |
| 151  | Cristalina              | GO    | 16°25’59” S, 47°35’39” W    | 881          | Soybean-sweet corn         |
| 152  | Cristalina              | GO    | 16°17’48” S, 47°37’38” W    | 975          | Soybean-fallow             |
| 153  | Cristalina              | GO    | 16°03’07” S, 47°24’29” W    | 927          | Soybean-fallow             |
| 154  | Planaltina              | DF    | 16°01’02” S, 47°26’11” W    | 954          | Soybean-fallow             |
| 155  | Planaltina              | DF    | 15°51’29” S, 47°23’14” W    | 882          | Soybean-wheat              |
| 156  | Planaltina              | DF    | 15°48’10” S, 47°38’16” W    | 1034         | Soybean-fallow             |
| 157  | Planaltina              | DF    | 15°43’29” S, 47°23’21” W    | 991          | Soybean-corn               |
| 158  | Planaltina              | DF    | 15°53’15” S, 47°23’29” W    | 890          | Soybean-bean               |
| 159  | Planaltina              | DF    | 15°57’48” S, 47°35’24” W    | 1020         | Soybean-millet             |
| 160  | Planaltina              | DF    | 16°00’29” S, 47°29’46” W    | 998          | Soybean-fallow             |
| 161  | Uberlândia              | MG    | 18°53’46” S, 48°17’28” W    | 865          | Standard population        |

*(1)Brazilian federative units: MG, state of Minas Gerais; GO, state of Goiás; and DF, Distrito Federal.*
the products for *Digitaria 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), intermediate 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, intermediate-resistant, and resistant, all weed populations were susceptible to clethodim; 97.5% were susceptible (Figure 1) and 2.5% intermediate-resistant to haloxyfop-P-methyl (Figure 2); and 9.9% were susceptible, 21.1% intermediate-resistant, and 68.9% resistant to glyphosate (Figure 3). The results confirmed the dispersion of glyphosate-resistant *Digitaria insularis* plants in the Cerrado biome, in Brazil. According to Takano et al. (2018), glyphosate-resistant *Digitaria insularis* populations can evolve through independent selections, contributing to an expressive dissemination of resistance in agricultural areas. However, despite the survival of *Digitaria 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 *Digitaria 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.
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

![Figure 2. Response of *Digitaria insularis* populations to clethodim: 100% susceptible (green).](image-url)
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

**Susceptible**
- 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)
- Campinorte, GO (1)
- Campo Alegre de Goiás, GO (3)
- Catalão, GO (4)
- Chapada Gaiúcha, MG (1)
- Conquista, MG (1)
- Cristalina, GO (21)
- Edeia, GO (1)
- Formosa, GO (1)
- Formoso, MG (3)
- Frutal, MG (1)
- Guaraupava, MG (1)
- Goiutaba, GO (2)
- Hidrolina, GO (2)
- Indiara, GO (1)
- Iral de Minas, MG (1)
- Itumbiara, GO (1)
- Jandaia, GO (1)
- Jataí, GO (8)
- Jovânia, GO (1)
- Lagoa Grande, MG (1)
- Monte Alegre de Minas, MG (1)
- Montividiu, GO (2)
- Morrinhos, GO (1)
- Orizona, GO (1)
- Padre Bernardo, GO (10)
- Palmeiras de Goiás, GO (2)
- Paracatu, MG (2)
- Paraíso, 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 (7)
- 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)
- Uberlândia, MG (5)
- Unaí, MG (15)
- Uruaçu, GO (2)

**Intermediately**
- Abadia dos Dourados, MG (1)
- Montividiu, GO (1)
- Padre Bernardo, GO (1)
- Rio Verde, GO (1)

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

Pesq. agropec. bras., Brasilia, v.55, e01570, 2020
DOI: 10.1590/S1678-3921.pab2020.v55.01570
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) | 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 |                |
|                 | IX    | 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, 42, 50, 51, 55, 65 |                |
|                 | 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
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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.

**Acknowledgments**

To Roni Amaro Bueno, for his assistance in conducting the present work.

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