Spatio-Temporal Distribution of *Digitaria insularis*: Risk Analysis of Areas with Potential for Selection of Glyphosate-Resistant Biotypes in Eucalyptus Crops in Brazil

Gabriela Madureira Barroso 1, Ricardo Siqueira da Silva 2, Danielle Piuzauna Mucida 1, Cláudia Eduarda Borges 2, Sabrina Rodrigues Ferreira 2, José Carlos Barbosa dos Santos 2, Hamurabi Anizio Lins 3, Vander Mendonça 3, Daniel Valadão Silva 3 and José Barbosa dos Santos 2

1 Department of Forestry, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina 391000-000, Minas Gerais, Brazil; danielle.piuzauna@ufvjm.edu.br
2 Department of Agronomy, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina 391000-000, Minas Gerais, Brazil; ricardo.ufvjm@gmail.com (R.S.d.S.); claudiaeduarda2013@hotmail.com (C.E.B.); sabrina.agroufvjm@gmail.com (S.R.F); agronomj@gmail.com (J.C.B.d.S.); jbarbosasantos2015@gmail.com (J.B.d.S.)
3 Department of Agronomic and Forestry Sciences, Universidade Federal Rural do Semi-Árido, Diamantina 391000-000, Minas Gerais, Brazil; hamurabi_a@hotmail.com (H.A.L.); vander@ufersa.edu.br (V.M.); daniel.valadao@ufersa.edu.br (D.V.S.)

* Correspondence: gabriela.madureira@ufvjm.edu.br

Abstract: The objective of this study was to model the spatio-temporal distribution of *Digitaria insularis* (L. L'Hérit) and analyze the risk of selection of glyphosate-resistant biotypes in eucalyptus cultivation in Brazil. Global data on the distribution of the specie were collected and associated with their ideal growth characteristics. The models were generated using Climex software, providing a predictive modeling technique. Biological data, species distribution, and climatic parameters were used to predict and map potential areas for the species of interest through the combination of growth and stress indices, giving rise to the Ecoclimatic Index (EI). The spatial distribution of *D. insularis* is predominantly in South and Central America and southern North America. The model had a good fit with the collected data and predicted higher EI values for tropical and subtropical regions, as was the case in Brazil. Species growth can occur throughout the year, with lower rates in winter, mainly in the country’s southern regions. Brazil has high climatic suitability for the occurrence of *Digitaria insularis*. Due to the climate suitability evidenced by the models and the expressive use of the same active ingredient, there is a risk of selecting glyphosate-resistant *Digitaria insularis* biotypes in eucalyptus cultivation areas.

Keywords: Climex; herbicides; modeling; plant health; weed; resistance

1. Introduction

The forestry sector has expanded significantly in recent years, emphasizing eucalyptus (*Eucalyptus L'Hérit*) plantations, the most cultivated forest species in Brazil [1]. This sector is responsible for the supply of various products. It has great economic importance for the country, with a Gross National Product (GNP) of R$ 86.6 billion, representing 1.3% of the Brazilian GNP and 6.9% of the industrial GNP [1]. Brazil’s planted eucalyptus forests are considered the most productive in the world [1].

As part of the technological advances in the production of this crop, the control of pests, diseases, and weeds is essential to maintain high productivity [2]. Weed control is a determining factor for the development and productivity of eucalyptus [3], with grasses being the most aggressive [4]. Among grasses, *Digitaria insularis* (L.) Fedde is one of the most problematic and has been considered the pest with the most significant phytosanitary risk for cultivating eucalyptus since 2018 [5].
The cultivation of eucalyptus requires the use of herbicides to control weeds, especially in the first three years of planting, where glyphosate is the most used [6]. However, pesticides can cause some impacts on the environment, such as contamination of soil, water, and non-target organisms [7–9]. In addition, the intensive use of the same product can select biotypes resistant to the herbicide [10], affecting the sustainability of natural areas close to the plantation areas and even areas planted with eucalyptus, requiring more significant interventions during the cultivation cycles.

The increase in biotypes of this species showing resistance to glyphosate has increased over the years in the agricultural sector [11], causing alert for growers of other cultures that use this product. Glyphosate is highly recommended for eucalyptus crops [2,12], which may favor resistance. Glyphosate-resistant biotypes make the use of this herbicide unfeasible, forcing its replacement by others with a high residual effect and that are more harmful to the environment [8]. Even with the recent expansion of the herbicide market for eucalyptus, the number of glyphosate-based formulations commercialized in Brazil corresponds to 60% of the total products available for the chemical control of weeds in this crop [12]. Edaphoclimatic conditions and the territorial extension of Brazil favor environments with variations in climate; in addition, climate change alterations affect agroclimatic zoning, including in the forestry sector. Tools that facilitate the prediction of occupation niches by pest species could be used for integrated management programs [13–18].

Climate is a determining factor for the growth of plants, as they require optimum amounts of temperature, light, and humidity. Several models relate climate to the spatial distribution of species, such as the generalized linear model, MaxEnt, random forest, boosted regression tree, and Bioclim [19–21]. Climex is a mechanistic bioclimatic model that predicts a species’ climatic suitability and growth according to climate and physiological characteristics [22]. Based on the model, it is possible to evaluate the development of the species over time [22], favoring decision-making for efficient management in the areas of most significant risk. In addition to determining the areas of most significant climatic adequacy, the model can be used as an alert for selecting herbicide-resistant plants, thus highlighting the novelty of the work.

The study hypothesizes that areas with favorable climatic conditions for the development of *D. insularis*, combined with the use of the same pesticide to control it, can select resistant biotypes in eucalyptus plantation areas. In this way, the objective of this study was to determine a model of the potential spatio-temporal distribution of *D. insularis* in Climex and to analyze the risk of glyphosate-resistant biotype selection in eucalyptus cultivation in Brazil.

2. Materials and Methods

2.1. Global Distribution of *Digitaria insularis*

The occurrence data for *D. insularis* were obtained from the Global Biodiversity Information Facility [23], European and Mediterranean Plant Protection Organization [24], and United States Department of Agriculture [25].

2.2. Climex

Climex is a mechanistic bioclimatic model that predicts the climatic suitability and growth of species, such as insect pests, weeds, and invasive species [22]. Climex integrates the weekly responses of a population to humidity and temperature and calculates annual indices from them using a set of adjusted growth and stress functions to assess the potential of a species to develop in a given location [22]. In this type of predictive modeling, biological data and species distribution, and climatic parameters are used to predict and map potential areas for the species of interest through the combination of growth and stress indices, which gives rise to the Ecoclimatic Index (EI) [22]. The EI ranges from 0 to 100, with 0 being a non-favorable location for the species’ survival and ≥30 denoting a very favorable climate for the species [22]. Furthermore, this modeling allows us to understand how species respond to climatic variables at different time scales (using the weekly growth
index, GIw). Thus, it is possible to estimate a species’ potential geographic distribution and seasonal suitability concerning the climate. The growth index (GI) represents the suitability of the site for growth and development and is calculated according to the proximity of environmental temperatures, soil moisture or day lengths to the ideals of growth of the species [22]. For seasonal adequacy, the software provides the GIw, which describes the favorable conditions for population growth on a scale of 0 to 1. Values near 0 indicate inadequate periods for species growth, whereas the closer the value to 1, the more favorable the conditions for the period [22]. The Ecoclimatic Index is: 

$$EI = TGI_A \times SI \times SX$$

where:

- $GI_A$, the Annual Growth Index

$$100 \sum_{i=1}^{52} TGI_{Wi}/52$$

- SI, the Annual Stress Index, = $(1 - CS/100) \times (1 - DS/100) \times SI \times (1 - HS/100) \times (1 - WS/100)$

- SX, the Stress Interaction Index, = $(1 - CDX/100) \times (1 - C \times SX \times WX/100) \times (1 - HDX/100) \times (1 - HWX/100)$

CS, DS, HS, WS are the annual cold, dry, heat and wet Stress indices respectively, and CDX, CWX, HDX and HWX are the annual cold–dry, cold–wet, hot–dry and hot–wet stress interaction indices.

2.3. Parameters Used for Model Creation and Validation

A total of 1840 occurrence points were obtained with geographic coordinates of *D. insularis* in the world, 1640 used to adjust the model, and 200 data recorded in Argentina were omitted and used for model validation [23–25]. The parameter values were the results of biological data of the species obtained from a literature review on *D. insularis*. Climatic data in CliMond 10’ grid were used to model in CLIMEX version 4.0. Minimum monthly average temperature (T min), maximum monthly average temperature (T max), monthly average precipitation (total P), and relative humidity at 09:00 h (RH 09:00) and 15:00 h (RH 15:00). Historical climatology averaged from 1961 to 1990 and finally averaged at 1975 [22]. The output has a relatively small horizontal grid spacing fulfilling the CLIMEX requirements.

2.3.1. Temperature Index

The thermal requirements of the species have previously been reported, and the results indicate a low-temperature threshold of 5 °C and a high value of 45 °C; temperatures greater than this would be lethal for the growth of *D. insularis* [26]. Thus, we used a low limiting temperature (DV0) of 5 °C and a high limiting temperature (DV3) of 45 °C. It has been shown that a temperature of 25 °C to 40 °C is favorable for the germination and growth of *D. insularis* [26]; therefore, we set the lowest optimum (DV1) and optimum upper (DV2) at 25 °C and 40 °C, respectively. In addition, *D. insularis* requires 1190 °C days for its complete development [27]. Thus, the PDD (degrees days) was adjusted to 1190 °C days.

2.3.2. Moisture Index

The moisture index’s optimum and maximum upper limits were established according to the global species distribution and were more prevalent in tropical areas. The optimal and minimum lower limits were defined according to the best fit of the model to the species distribution data. Thus, we set the lower limit of soil moisture (SM0) to 0.008 and the upper limit (SM3) to 2.5. Our values for the lower limit of ideal soil moisture (SM1) and optimal upper threshold (SM2) were 0.02 and 1.5, respectively.

2.3.3. Stress Indices

The cold stress index (DTCS) was determined based on the lower growth limit of the species, which was 5 °C with an accumulation rate (DHCS) of −0.001 week⁻¹, and the
upper limit of 45 °C was used for the heat stress index (TTCS) with an accumulation rate (THCS) of 0.0002 week^{-1} [26]. We established our soil moisture level limit for dry stress (SMDS) at 0.001 and its accumulation rate (HDS) at −0.0001 week\(^{-1}\). These values were used because of the high tolerance of *D. insularis* to low humidity and its distribution in arid climates [22]. However, since excess moisture is not a limiting factor for the development of this species, we set our wet stress parameter (SMWS) at 2.5, with the accumulation rate (HWS) at 0.002 week\(^{-1}\). These parameters were also chosen to present the best possible fit to the data collected from the actual distribution of *D. insularis*.

2.3.4. Meteorological Data

The first model was created to compare the locations over a year for *D. insularis*; therefore, it was necessary to use a monthly time series of climatic data. Thus, CRU TS3.23 was used: Climate Research Unit (CRU) Time-Series (TS) Version 3.23 with data in a high-resolution grid of month-to-month climate variations. CRU TS 3.23 reformatted the data for the variables required by Climex, such as precipitation, the monthly average of the maximum and minimum daily temperature, and steam pressure from January 1901 to December 2017. All output files of CRU TS were the actual values. For the growth index models throughout the year, we executed our Climex model from 1 January 2016 to 31 December 2016. The model was also generated for the 10 years before the first case of resistance from *D. insularis* to herbicides using the coordinates of the city of Guaíra, Paraná, Brazil, where the first report occurred in the year 2008 [28]. These data were used to evaluate the possible favoring of climatic conditions for developing the species, which, together with herbicides, can be applied to select resistant biotypes.

2.4. Model Verification and Validation

In the verification step, the initial model was based on distributions of *D. insularis* in Brazil. After minor adjustments to CLIMEX parameters, most distributions were modeled as having optimal conditions for *D. insularis*. After that, the model was validated by comparing the output to known distributions of *D. insularis* in South America. These model verification and validation results demonstrate realistic estimations and reliability in the final model.

2.5. Eucalyptus Cultivation Versus Herbicide Consumption

To relate the risk of selecting herbicide-resistant biotypes in eucalyptus crops, a database was created on the area planted and the amount of herbicide sold by the state in Brazil.

3. Results

There were 1840 points of occurrence of *D. insularis* with geographical coordinates worldwide, including 614 in Mexico, 289 in Brazil, 200 in Argentina, 139 in Paraguay, 58 in Ecuador, 53 in the USA, 48 in Nicaragua, 39 in Colombia, 32 in Bolivia, 35 in Puerto Rico, 36 in Honduras, and others with fewer occurrences (Figure 1). Of the 289 occurrence points recorded in Brazil, the highest numbers were in Bahia, Goiás, Mato Grosso do Sul, and São Paulo (Table 1).

The potential distribution model of *D. insularis* showed an excellent fit to the actual occurrence data collected. More than 99% of the occurrence points were within the areas predicted by the model as suitable for the development of the species. The most climatic suitability worldwide was observed in tropical and subtropical regions, emphasizing South and Central America. In Brazil, EI values greater than 30 were observed in all states (Figure 2).
Figure 1. Map of the geographical distribution of Digitaria insularis in the world with emphasis on Brazil.

Table 1. Points of occurrence of Digitaria insularis in the Brazilian states.

| States         | Records of D. insularis | States          | Records of D. insularis |
|----------------|-------------------------|-----------------|-------------------------|
| Acre           | 0                       | Paraíba         | 10                      |
| Alagoas        | 3                       | Parana          | 17                      |
| Amapá          | 0                       | Pernambuco      | 14                      |
| Amazonas       | 8                       | Piauí           | 3                       |
| Bahia          | 54                      | Rio de Janeiro  | 7                       |
| Ceará          | 1                       | Rio Grande do Norte | 9                   |
| Espírito Santo | 3                       | Rio Grande Sul  | 13                      |
| Goiás          | 25                      | Rondônia        | 1                       |
| Maranhão       | 11                      | Roraima         | 1                       |
| Mato Grosso    | 18                      | Santa Catarina  | 15                      |
| Mato Grosso Sul| 23                      | São Paulo       | 21                      |
| Minas Gerais   | 10                      | Sergipe         | 13                      |
| Pará           | 7                       | Tocantins       | 2                       |

The values of the growth index of D. insularis approached 1 in most of the Brazilian territory throughout the year. In May, June, July, and August, lower growth index values were observed in the extreme south of Brazil and the central and western regions of the country (Figure 3).

The area planted with eucalyptus in Brazil and the consumption of herbicides by state is shown in Figure 4. The largest planted areas are in the states of Minas Gerais, São Paulo, Mato Grosso do Sul, Bahia, and Rio Grande do Sul, and the greatest use of herbicides are in the states of Mato Grosso, São Paulo, Rio Grande do Sul, Goiás, and Mato Grosso do Sul. Among the most commercialized herbicides, glyphosate is dominant, representing more than 58% sales (Figure 4).

The growth rate evaluated during the years prior to the first report of resistance of D. insularis to glyphosate showed a sharp drop in the winter months in Brazil. In the months of spring and summer, the values remained at the maximum; however, there was no significant variation in growth rates over the period evaluated, with averages between 1.0 and 0.76 (Figure 5).
Figure 2. Climatic adaptation for *Digitaria insularis* in the world and especially in South America based on the Climex model. Values of EI = 0 (not suitable), 0 < EI < 30 (suitable), EI ≥ 30 (highly suitable).

Figure 3. Cont.
Figure 3. Monthly variation in the growth index (GI 0 to 1) made in Climex for *Digitaria insularis* in South America in the period from 1 January to 31 December 2016. More reddish areas correspond to GI values closer to 1, more blueish tones are equivalent to GI values closer to 0.

Figure 4. Overlapping area planted with eucalyptus in Brazil and the sale of herbicides by state, in 2019, highlighting glyphosate close to 60% of the total. Source of data: [1,29].
The growth rate evaluated during the years prior to the first report of resistance of *D. insularis* to glyphosate showed a sharp drop in the winter months in Brazil. In the months of spring and summer, the values remained at the maximum; however, there was no significant variation in growth rates over the period evaluated, with averages between 1.0 and 0.76 (Figure 5).

Figure 5. The growth index of *Digitaria insularis* projected during the ten years before the first case of resistance to herbicides of the species in Brazil in the city of Guaíra–PR (First report in 2008).

4. Discussion

Grasses are the most aggressive weeds in eucalyptus cultivation [4], and in Brazil, the most significant infestations by *D. insularis* coincide with some of the largest planted areas of this crop (Figure 4). The Ministério da Agricultura, Pecuária e Abastecimento (MAPA), using Normative Instruction No. 112 (8 October 2018), decreed *D. insularis* as a priority pest of phytosanitary and economic risk for the eucalyptus crop because of its wide distribution and challenging control [5]. This species has herbicide-resistant biotypes in Brazil, with an
increasing number identified in agricultural areas over the past few years [11], making it potentially problematic in forest areas with eucalyptus cultivation.

Grass species have a wide global distribution, which vary according to their physiological and morphological characteristics. Species of the genus Miscanthus, for example, are distributed in regions of Japan, Korea and China [30]. Another example is Hyparrhenia hirta, which occurs mainly in regions with a subtropical, warm temperate or Mediterranean climate [31]. On the other hand, the distribution of D. insularis is broad and its native areas are South and Central America, and southern North America [32]. This distribution mainly in tropical and subtropical regions is due to physiological characteristics, such as its photosynthetic type C4 metabolism, which is dependent on a more significant amount of energy for development, with optimal temperatures between 25 °C and 35 °C [26]. Thus, countries with a tropical climate, such as Brazil, are conducive to the growth and multiplication of D. insularis, with optimal growth temperatures between 25 °C and 40 °C [26]. In tropical climates, the soil seed bank of D. insularis is continually renewed. This species produces seeds throughout the year and at temperatures that allow for continuous germination and development [33]. In addition, it can reproduce both seeds and rhizomes, which facilitates its dispersion, mainly in agricultural areas [34].

Predictive modeling using the Climex software is an effective tool for analyzing the climatic suitability of plants in a given location using climatic and physiological variables [35]. The models can be used to make decisions regarding managing the species of interest for agricultural crops and invasive species [36,37]. The most excellent climatic suitability for the development of the species was observed precisely where there were more points of occurrence (Figure 1), which are the places where climatic conditions are favorable to the species’ growth. It is not possible to state that all occurrence points are shown in Figure 1, but the modeling uses these points where the occurrence of the species has already been reported to validate the models. This is because the databases used have georeferenced reporting points for the species. These data are used to compare the areas where the species is known to occur and the areas where the model predicts high climatic suitability for the species to occur. If the model does not predict adequacy in an area where the species already occurs, the model is not valid. EI values greater than 30, observed throughout the Brazilian territory, show that the country has good conditions for developing D. insularis.

In addition to predicting climate suitability, it is crucial to analyze the species’ growth index (GI). The values of this index close to 1 observed in Brazil throughout the year indicate favorable conditions for the population growth of D. insularis in most seasons. The closer this index is to 1, the more favorable the conditions of the period [22]. The lowest values observed in May, June, July, and August coincide with the Brazilian winter period, where temperatures are lower, particularly in the south, reaching −8.6 °C. As the optimal temperatures for the species’ growth vary between 25 °C and 40 °C [26], cold stress reduces the development of the species during this period.

Brazil has experienced a significant expansion of eucalyptus crops in recent years, with yields of approximately 36 m³ ha⁻¹ year⁻¹ in 2019 [1]. This high productivity can be compromised by the incorrect chemical management of weeds over time. As in agricultural areas, only one active herbicide ingredient can cause resistant weeds and make control more difficult. Some of the states with the most eucalyptus plantations also had the highest consumption of herbicides (Figure 4). Resistance mainly occurs due to selection pressure, given by the increase in the population of plants and the use of the same product, which in Brazil is glyphosate. The high climatic suitability predicted by the model for developing D. insularis throughout the country, combined with the high growth rates and glyphosate consumption, generates an increased risk of selecting resistant biotypes in eucalyptus crops. According to the recommendations for the crop, applications can reach 20 L ha⁻¹ [2], which would be equivalent to 114 million liters of glyphosate used in the cultivation of eucalyptus.

Climex modeling, in addition to generating predictive models to guide future perspectives, can be used to understand events in the past [22]. This type of modeling can be used
as a crop management tool, indicating suitable areas for cultivation in the face of climate change or analyzing the risk of pest invasion [38,39]. When evaluating the history in the city where the first report of *D. insularis* resistant to glyphosate occurred, we noticed the same general behavior of the seasonal growth of the species, with values close to one and a fall in the winter months. There were no significant variations in growth rates over time, that is, there was no climatic favoring for greater growth of the *D. insularis* population, which could be related to the selection of the resistant biotype in 2008 [28].

The forestry sector has the advantage of low disturbance, reducing the infestation of weeds. However, eucalyptus cultivation is characterized by solid weed growth in the first three years after planting, when the area between the lines is not protected by shading. Chemical control with herbicides is the most widely adopted method of weed reduction, with a pre-emergent application generally made before planting and an annual direct application after planting [2].

Despite presenting a trend of a proportional decrease in the glyphosate applied per unit area, the current scenario still prioritizes this active ingredient. In Brazil, 871 commercial herbicide brands are registered, 113 containing glyphosate as an isolated or mixed ingredient [12]. Considering only the forestry sector, 72 of the 139 herbicide formulations registered for eucalyptus (51.8%) contained glyphosate. Despite the growing recommendation to use pre-emergent herbicides, glyphosate is still the main post-emergent chemical control option for grasses [12]. Therefore, this reinforces that only climatic adequacy and use of the same active ingredient are sufficient for selecting the biotype of the species resistant to glyphosate. This fact strengthens the risk of the same event in areas with eucalyptus cultivation, which are highly suited to the occurrence of the species and remain under constant application with glyphosate.

5. Conclusions

Brazil has high climatic suitability for the occurrence of *Digitaria insularis*, and the growth of this species can occur throughout the year, with lower rates in winter, especially in the south of the country.

Due to climatic suitability and the expressive use of the same active ingredient, there is a risk of selecting glyphosate-resistant *Digitaria insularis* biotypes in eucalyptus cultivation areas.

The management of the species with herbicides must consider the entire year, since the conditions for the species’ growth are favorable during all months of the year, as evidenced by the Climex model.

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