

## Article

# Population Dynamics of *Digitaria sanguinalis* and Effects on Soybean Crop under Different Glyphosate Application Timings

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**Abstract:** Large crabgrass (*Digitaria sanguinalis*) is one of the most problematic weeds in summer crops in Argentina. Emergence throughout the season of several cohorts allows the weed to escape postemergence control. Demographic models are useful tools to understand and compare the effect of different agronomic management decisions on weed population growth, as well as to identify critical functional stages that affect population growth rates. The objectives of this work were (i) to study population dynamics of *D. sanguinalis* in soybean, (ii) to determine the effect of glyphosate application timing on weed demographic parameters and soybean yield losses, and (iii) to evaluate the effect of weed density on soybean yield loss. A field experiment was conducted in two locations, in a completely randomized design with three replicates. Treatments included a control without glyphosate and glyphosate applied at soybean stages V4 or R1. The demographic stages (initial seedbank, seedlings, and adult plants) and parameters (establishment, survival, and fecundity) were estimated. Reproductive organs were evaluated in each cohort, including raceme per plant, spikelets per raceme, and seeds per spikelet. Weed and crop biomass and yield crop were assessed at harvest. Three cohorts were identified, the first of which emerged in November and contributed 93% of the total seedlings and 71% of the total adults. Glyphosate applied at V4 reduced the survival rate of the first cohort, as well as the total shoot biomass and the fecundity rate, increasing the biomass and crop grain yield. Both application timings affected tillers per plant, racemes per tiller, and fertile spikelets per raceme. Glyphosate at R1 did not effectively reduce weed competition, but reduced seed production as application at V4. Yield losses estimated with the model of the rectangular hyperbola according to weed density showed a yield loss at low densities (I) of 18%, and a maximum yield loss (A) of 82%. To avoid yield losses, herbicide applications targeting the first cohort are more effective than later applications targeting subsequent cohorts. However, at both times glyphosate applications reduced the number of seeds entering the seedbank, and therefore the population growth rate.

**Keywords:** crop-weed competition; demographic stages; fecundity rate; germination rate; large crabgrass; population growth rate; survival rate; weed density; yield loss



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## 1. Introduction

Large crabgrass (*Digitaria sanguinalis* (L) Scop., family *Poaceae*), is an annual grass that is native to Europe and distributed throughout all tropical and temperate regions around the world [1]. In Argentina, it was considered one of the 10 most important weeds when conventional tillage was the main system used [2]. Since the mid-1990s, the adoption of no-tillage systems increased steadily, occupying over 90% of the country's agriculture area due to simplicity and the low cost of genetically modified glyphosate-resistant soybean production [3]. Despite the fact that glyphosate is 98–100% effective at controlling *D. sanguinalis* [4,5], large crabgrass has maintained or even increased its abundance and constancy in no-tillage systems [6–9]. In addition, a survey showed that it was the only non-herbicide resistant or glyphosate-tolerant species considered among the

10 most important weeds in Argentina [2]. However, recently, the first glyphosate resistant biotype was registered in the country [10].

Some characteristics of large crabgrass could explain the success of this weed; for example, its high seed production [11] and the preference of broadleaf seeds over grass seeds by seed predators in no-tillage systems [12]. Probably the most important characteristic is an emergence distributed throughout the season in three to five cohorts [13,14], which allows the weed to escape control. The use of residual herbicides, such as metolachlor, acetochlor, and pendimethalin, showed control levels of 100%, 91%, and 83%, respectively [15,16]. However, herbicide efficacy is highly dependent on soil characteristics and environmental conditions during post application [17,18], which are extremely variable from spring to mid-summer, during the emergence window of this species [13,14,19].

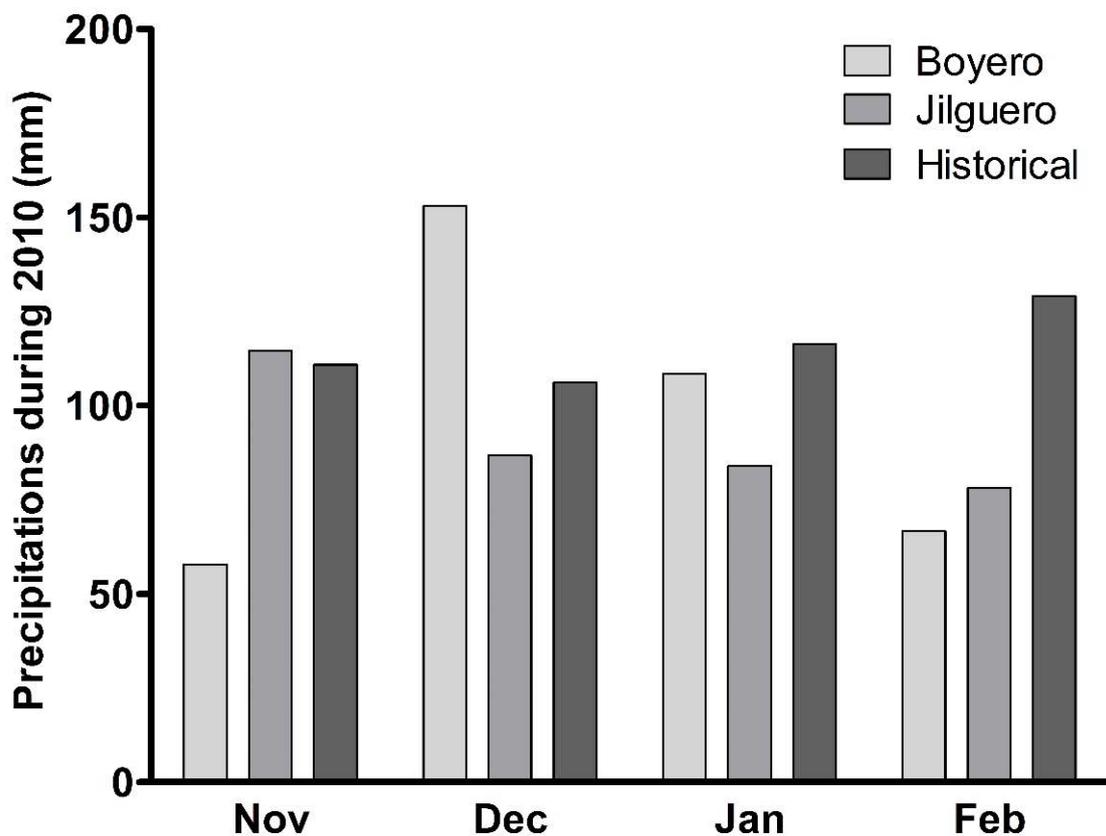
Identifying the critical period for weed control (CPWC) is important for integrated weed management (IWM) [20]. The CPWC is the period in the crop life cycle when weed control is necessary to prevent yield loss. In soybean, this period is approximately V2–V4, and sometimes R3, which is too short to consider the use of residual herbicides [21]. On the other hand, the presence of those weeds emerging after the CPWC could have no effect on the yield, but can contribute to renewal of the seedbank to maintain weed populations [22]. However, sequential postemergence applications to control every single cohort as it emerges is not recommended due to the increasing crop costs, higher risk of environmental pollution [23], and evolution of herbicide-resistant biotypes [24], as well as selection of a few species that become dominant and difficult to manage [25].

Demographic models are useful tools to understand and compare the effect of different agronomic management decisions on weed population growth rates [26], and to identify critical demographic processes that have more impact on population growth rates [27]. Therefore, to make successful weed management decisions, it is necessary to know not only the biology and ecology of the weeds, but also the effects of management practices on population dynamics and demographic processes such as emergence, survival, and fecundity [28,29]. The objectives of this work were (i) to study *D. sanguinalis* population dynamics in soybean, (ii) to determine the effect of glyphosate application timing on weed demographic parameters and soybean yield losses, and (iii) to evaluate the effect of weed density on soybean yield loss.

## 2. Materials and Methods

### 2.1. Experimental Site

The study was conducted in two commercial soybean fields (Boyero and Jilguero), in 2010, naturally and homogeneously infested with *D. sanguinalis*, under no-tillage systems, separated by 5 km in the district of Salto, province of Buenos Aires (34°25' S, 60°15' W), in the centre of the Rolling Pampas. This region has a temperate-humid climate with hot summers, an average annual rainfall of 950 mm, and a mean annual temperature of 17 °C [30]. The soil in both locations was Argiudol. Before the experiment, a 0–20 cm layer of the soil was analysed; Boyero had 2.82% organic matter, 6.24 pH, and 3.64 NO<sub>3</sub> kg<sup>-1</sup> (total by Kjeldahl), and Jilguero had 3.14% organic matter, 6.03 pH, and 5.54 NO<sub>3</sub> kg<sup>-1</sup> (total by Kjeldahl). Rainfall was registered monthly at each location (Figure 1).



**Figure 1.** Monthly rainfall in locations Jilguero and Boyero, and historical rainfall during the months that the experiment was performed in the field.

### 2.2. Experiments

A completely randomized experimental design, with three replicates, was established in each location. Treatments were: (i) control without glyphosate, (ii) application of glyphosate at soybean stage V4, and (iii) application of glyphosate at soybean stage R1. The main plot had a size of 16 m × 16 m (256 m<sup>2</sup>).

Glyphosate-resistant (Roundup Ready<sup>®</sup>) soybean cultivar (“Don Mario 4800<sup>®</sup>”) (Don Mario Semillas, Chacabuco, Buenos Aires, Argentina) was planted at 42 plant m<sup>-2</sup> and 52 cm interrow spacing. The sowing date was 3 November in Boyero and 25 November in Jilguero. Seeds were inoculated with *Bradyrhizobium japonicum* (Nitragin Optimize<sup>®</sup>, 3 mL kg<sup>-1</sup> seed, 753 10th Street, Pilar, Buenos Aires, Argentina). Glyphosate was applied at 2000 g ae ha<sup>-1</sup> in the entire field before sowing.

Three sub-samples of soil per treatment and replicate were taken randomly to evaluate the number of seeds in the seedbank. These samples were taken using an aluminium cylinder with a 5 cm diameter buried at a depth of 5 cm. The samples were aerated to remove excess moisture, then sieved, and *D. sanguinalis* seeds were identified and counted. Glyphosate was applied (1000 g ae ha<sup>-1</sup>) using ground-based spraying equipment with flat-fan nozzles (AIXR 11002 TeeJet<sup>®</sup>) calibrated to deliver 80 L ha<sup>-1</sup> at 152 kPa. The application at V4 occurred 14 days after the first cohort was registered (33 and 29 days after crop emergence in Boyero and Jilguero, respectively), and the application at R1 occurred 14 days after the second cohort was registered (65 and 55 days after crop emergence in Boyero and Jilguero, respectively).

### 2.3. Measurements

Every 30 days, seedling density was evaluated in three quadrats (20 cm × 20 cm) randomly located in each plot. Seedlings were marked with wires of different colours for each cohort and counted. At harvest, plants were classified into dead and alive adult plants,

with and without seeds, in every cohort. The number of individuals per category, tillers per plant, and fertile spikelets per tiller were registered for each cohort. Finally, total aerial biomass of the weed was collected and dried at 70 °C until constant weight was achieved, after which samples were weighed.

At harvest, weed density, crop total aboveground biomass, and yield were also evaluated by collecting three random samples per plot, equivalent to 1 m<sup>2</sup>. The samples were placed in an oven at 70 °C until weight was constant, and then weighed.

A demographic approach was used to study the population dynamics of *D. sanguinalis* in soybean crops [31,32], and a life table corresponding to each treatment was made. The demographic stages (initial seedbank, seedlings, and adult plants) and parameters (establishment, survival, and fecundity) were estimated.

The rate of emergence was estimated according to Equation (1), where  $Er$  is the emergence rate:

$$Er = (\text{Total seedlings emerged})/(\text{Seeds in the seedbank}) \quad (1)$$

The rate of survival was estimated for each cohort according to Equation (2), where  $Sr$  is survival rate:

$$Sr = \text{Adult plants}/\text{Emerged seedlings} \quad (2)$$

The fecundity rate (number of seeds per plant) was estimated for each cohort according to Equation (3), where  $Fr$  is fecundity rate:

$$Fr = \text{Tillers per plant} \times \text{raceme per tiller} \times \text{fertile spikelets per raceme} \quad (3)$$

To evaluate crop performance as a function of *D. sanguinalis* density ( $D$ ), the yield values were transformed into percentage values relative to the average value from plots with glyphosate applied at V4 without weeds, and adjusted to the nonlinear regression model of the rectangular hyperbola proposed by [33] Equation 5:

$$Yl = (I.D)/(1 + I/A.D) \quad (4)$$

where  $Yl$  is yield loss (%),  $D$  is weed density,  $I$  is the yield loss per unit of weed when weed density approaches zero, and  $A$  is yield loss when weed density tends to infinity, respectively.

#### 2.4. Data Analysis

The results of each treatment were assessed separately by analysis of variance (ANOVA), with a significance level of 95%. The number of seeds in the initial seed bank, the number of seedlings in each cohort, the number of adult plants in each cohort, the number of total tillers per adult, the number of racemes per tiller, the number of fertile spikelets per raceme, weed biomass, soybean biomass, and yield were registered and subjected to ANOVA followed by Tukey's multiple comparison test using a general linear model procedure in R [34]. The data were square root transformed when ANOVA assumptions were not met (random sampling, homoscedasticity, and normal distribution of residuals). No differences were observed in demographic data (initial seed bank, number of seedlings in each cohort, and number of adult plants) or the respective rates between locations (Boyero and Jilguero) (Table 1). Therefore, data were merged and analysed together. The same was observed for aboveground biomass and crop yield. In plant characteristics, such as total tillers per plant, racemes per tiller, and fertile spikelets per raceme, there were some differences between locations. Therefore, data were presented separately.

**Table 1.** Data of demographic stages (seeds in the seedbank, seedlings, and adult plants per m<sup>2</sup>) and parameters (emergence, survival, and fecundity rates) of *D. sanguinalis* for the different treatments, no glyphosate, glyphosate applied in V4, and in R1. Emergence rate (*Er*), survival rate (*Sr*), and fecundity rate (*Fr*). Values are the average from both locations (Jilguero and Boyero). Uppercase letters in the same rows mean significant differences among treatments according to Tukey's multiple comparison test ( $p < 0.05$ ). Lowercase letters in the same rows mean significant differences among each cohort ( $p < 0.05$ ).

	No Glyphosate			Glyphosate at V4			Glyphosate at R1		
Initial seedbank	7037A			5961A			6893A		
<i>Er</i>	0.17A			0.11A			0.15A		
Cohort	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Seedlings	1175Aa	167Aab	0Ab	621Aa	17Ab	25Ab	721Aa	200Aab	88Ab
<i>Sr</i>	0.42Aa	0.22Aa	0Aa	0Ba	0Aa	0.5Aa	0.19Aba	0.42Aa	0.40Aa
Adult plants	388Aa	29Ab	0Ab	0Ba	0Aa	17Aa	150Aba	113Aa	63Aa
<i>Fr</i>	1556.2Aa	0.5Ab	0Ab	0Ba	0Aa	1.7Aa	3.4B	0.25A	0.5A

### 3. Results

#### 3.1. *Digitaria Sanguinalis* Population Dynamics

In both locations (Jilguero and Boyero), three cohorts were identified, and peak seedling emergence was registered during November. At this time, 75–96% of the total seedlings had emerged in all treatments, except for plots where glyphosate was applied at R1 in Boyero, where only 63% had emerged. The rest of the cohorts emerged in late December and January, and both were significantly lower ( $p < 0.05$ ) than the first cohort. No differences were observed among treatments and locations in emergence rates or the number of seeds in the seedbank at the beginning of the experiment. However, the emergence rates tended to be higher without controls than with glyphosate applications, and in Boyero than in Jilguero, independently of the treatment (Table 1).

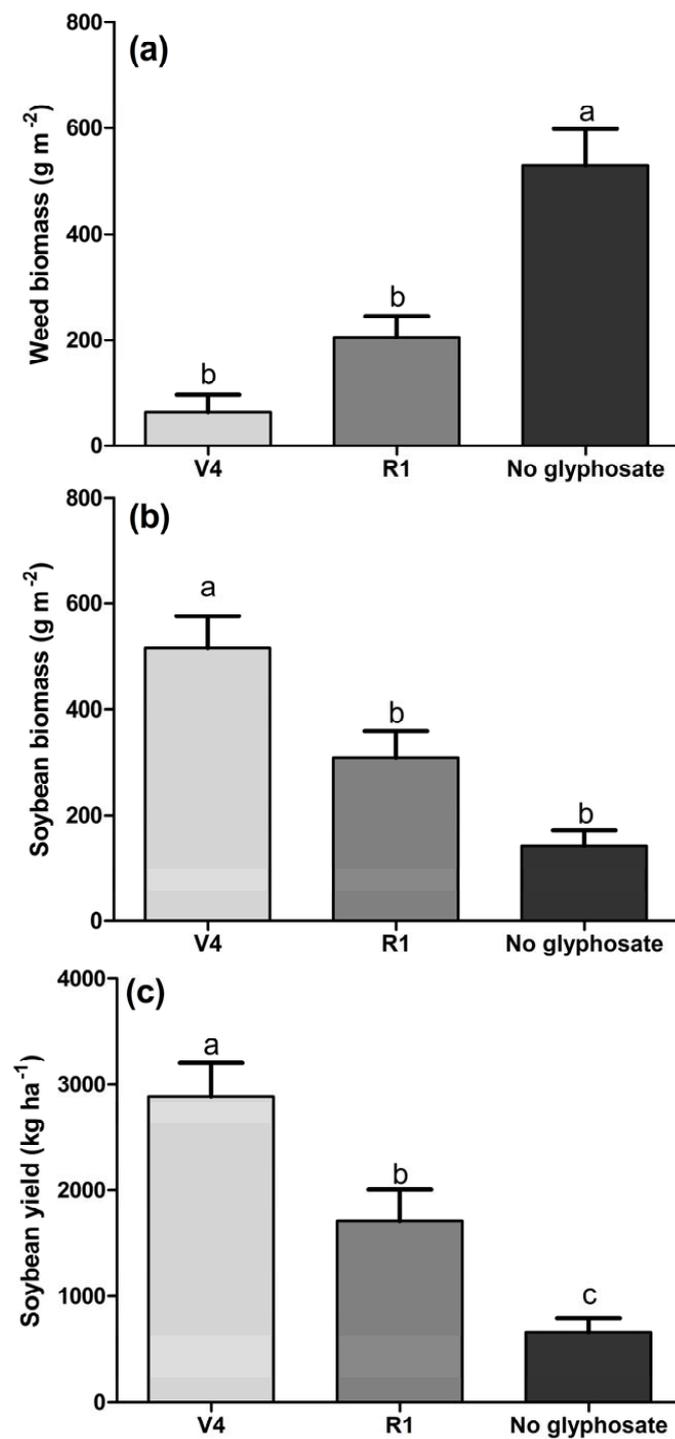
There were no differences among treatments in the number of seedlings from the same cohort in any location. However, there were differences ( $p < 0.05$ ) among cohorts depending on the treatment. Independent of location, without glyphosate or with glyphosate applied at R1, the number of seedlings was higher in the first cohort than in cohort three, and with glyphosate at V4 the first cohort had more seedlings ( $p < 0.001$ ) than the other two (Table 1). For the first cohort, the survival rate was lower ( $p < 0.001$ ) with glyphosate at V4 than without glyphosate in both locations, but no differences among treatments were observed in the other cohorts. In addition, no differences were observed among cohorts for the same treatment in any location. In all the treatments, the number of adult plants in the first cohort tended to be higher than the rest of the cohorts, and no differences were observed among treatments and cohorts in both locations.

There was a higher ( $p < 0.0001$ ) fecundity rate without glyphosate than with glyphosate in both locations. Without glyphosate, the first cohort had a higher number of seeds per plant than the second and third cohorts. In both treatments with glyphosate, no differences were observed among cohorts in either location (Table 1).

In the first cohort, a higher number of total tillers per plant was observed without glyphosate than with glyphosate at V4 in Boyero, which was also higher than the application at V4 and R1 in Jilguero ( $p < 0.05$ ). In Jilguero, without glyphosate, the second cohort had more tillers per plant than with glyphosate ( $p < 0.001$ ). No differences were observed in the second and third cohorts in Boyero, or in the third cohort of Jilguero (Table 2).

In Jilguero, the first and second cohort had a higher number of racemes per tiller without than with glyphosate ( $p < 0.001$ ). In Boyero, differences were observed only in the first cohort, with a higher number of racemes per tiller without glyphosate at V4 ( $p < 0.01$ ) (Table 2). As for the rest of the reproductive structures, a lower ( $p < 0.001$ ) number of spikelets due to the herbicide application was observed in the first cohort, both for Boyero

and Jilguero (Table 2). In both locations, there was a reduction ( $p < 0.05$ ) of shoot biomass when glyphosate was applied at V4 and R1 (Figure 2a).



**Figure 2.** (a) *D. sanguinalis* biomass (g m<sup>-2</sup>), (b) soybean biomass (g m<sup>-2</sup>), and (c) crop yield (kg ha<sup>-1</sup>) with glyphosate applied when soybean crop was at V4 stage (vegetative with 4 leaves) and R1 stage (reproductive stage with 1 flower), and without glyphosate application (no glyphosate). Values are the means, and vertical bars are SEM. Columns with the same letters are not significantly different according to Tukey's multiple comparison test ( $p < 0.05$ ).

**Table 2.** Number of total tillers per plant, racemes per tiller, and fertile spikelets per raceme of *D. sanguinalis*, in Boyero and Jilguero for the different treatments; no glyphosate, glyphosate applied at V4, and at R1. Different letters indicate significant differences among treatments for each location according to Tukey's multiple comparison test ( $p < 0.05$ ).

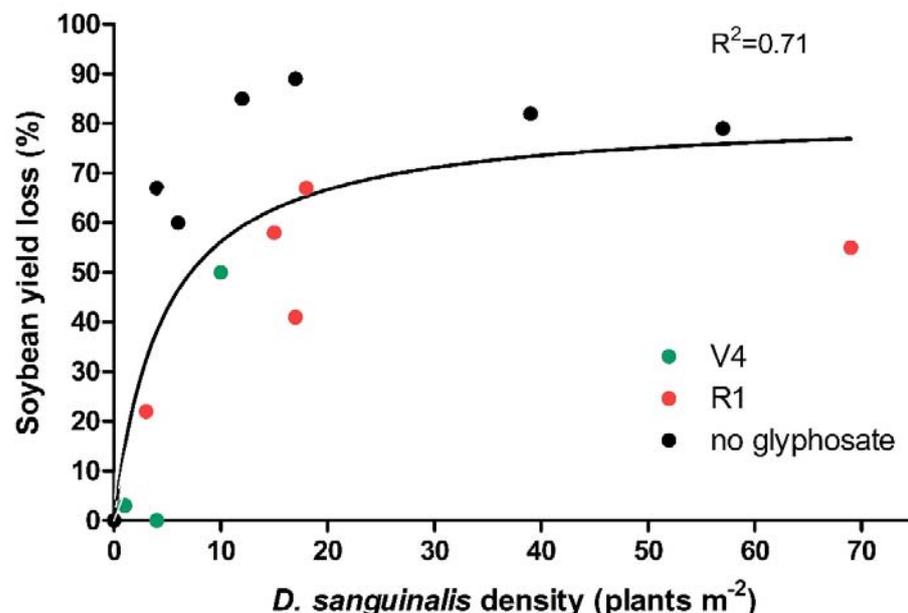
Boyero		Treatments								
		No Glyphosate			Glyphosate at V4			Glyphosate at R1		
Cohort		1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Tillers per plant	7.03b	0.5a	0a	0a	0a	0a	0.67a	1.58ab	0.5a	0.67a
Racemes/tiller	4.06b	0.5a	0a	0a	0a	0a	1a	1ab	0.42a	0.5a
Spikelets/raceme	30.89b	3a	0a	0a	0a	0a	5a	4.25a	2.42a	3a

Jilguero		Treatments								
		No Glyphosate			Glyphosate in V4			Glyphosate in R1		
Cohort		1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Tillers/plant	14.25b	2.67b	2.67a	0a	0a	0a	0a	0a	0a	0a
Racemes/tiller	4.75b	2.67a	1.33a	0a	0a	0a	0a	0a	0a	0a
Spikelets/raceme	33.25b	14.67a	9.33a	0a	0a	0a	0a	0a	0a	0a

### 3.2. Effects on Soybean

In both locations, soybean biomass production was higher ( $p < 0.001$ ) when glyphosate was applied at V4 than at R1 or without glyphosate (Figure 2b). The application of glyphosate at V4 showed higher yield than at R1 and without glyphosate ( $p < 0.05$ ), and glyphosate applied at R1 had a higher yield than without glyphosate (Figure 2c). Soybean yield loss increased asymptotically ( $R^2 = 0.68$ ) as the weed density (plants  $m^{-2}$ ) increased (Figure 3). The parameters of the model were estimated as 18% yield loss when density approaches zero ( $I$ ), and as 82% maximum yield loss at higher densities ( $A$ ).



**Figure 3.** Soybean yield loss (%) related to *D. sanguinalis* density (plants  $m^{-2}$ ) at different treatments, with glyphosate applied when soybean crop was at V4, at R1, and without glyphosate application (no glyphosate). The slope ( $I$ ) represents the percentage yield loss as density approaches zero, and the asymptote ( $A$ ) represents the percentage yield loss as density approaches infinity. Equation  $YI = 17.88 \times D / [1 + (17.88/82.06) \times D]$ ;  $R^2 = 0.71$ ;  $SS = 5156$ ;  $df = 15$ .

#### 4. Discussion

The life table of *D. sanguinalis* is similar to others described for all annual species that reproduce by seed [31], and results in a model similar to that presented for *Avena sterilis* and *Lolium rigidum* [28,34]. Despite the large number of seeds present in the seedbank (around 6130 seeds m<sup>-2</sup>), a very low proportion of them (between 8 and 35%) reached the seedling stage, regardless of the location or treatment. This is probably the result of crop and stubble presence in no-till systems, which have been shown to modify the environmental conditions surrounding the seeds, such as reducing temperature fluctuation and increasing far-red light [19]. *D. sanguinalis* seeds require fluctuating temperatures to terminate dormancy [35], and environments enriched with far-red light can reduce germination percentage [35]. In addition, as was observed in previous works from no-till systems with summer crops such as maize [36] or late soybean [37], there were three cohorts emerging in the season, which allowed the species to escape the postemergence applications and produce seeds. Among these cohorts, the first was the most relevant, and had the highest number of seedlings. This was the result of crop growth that reduced the solar radiation reaching the soil and the light quality as the season advanced [38,39], as well as *D. sanguinalis* plants themselves that had previously emerged, especially in the treatment without glyphosate.

The survival rate of the first cohort was reduced by glyphosate, especially when the herbicide was applied at the V4 stage, which reduced the number of adult plants. This early control of the first cohort allowed the crop to capture resources and increase its competitive ability against the next cohorts. Typically, the last cohorts are less competitive, and produce less biomass and seeds, than cohorts emerging earlier in the season [40,41]. When glyphosate was applied at R1, the survival rate and number of adults tended to be lower, but no differences were observed compared with no application. During the glyphosate application at R1, some of the plants from the first cohorts were in advanced stages of growth, which reduced glyphosate efficacy [42,43]. In addition, at R1, the interrow is more covered by the crop canopy than at V4 [39], which intercepts the herbicide and reduces its control efficacy [44]. Without glyphosate, later cohorts are not only exposed to crop competition, but also to intraspecific competition with *D. sanguinalis* plants that emerged earlier and are more competitive [45,46].

Glyphosate applications reduce seed production in *D. sanguinalis* not only by reducing the potential plants that can produce new seeds, but also by reducing the growth and fecundity of surviving plants. These surviving plants are less competitive in capturing resources that are taken by the crop, as they generate less biomass, allocate fewer resources to reproductive organs, and reduce their fecundity [47]. This generates a lower number of tillers per plant, racemes per tiller, and fertile spikelets per raceme produced by plants in treatments with glyphosate compared the controls. As a result, fewer plants produce seeds, and surviving plants produce fewer seeds. The application at V4 produced very few new seeds to enter to the seedbank, but the application at R1 had some escapes that produced new seeds in Boyero.

As the use of herbicides increased the competitive ability of the crop [48], this allowed the crop to capture more resources and produce more biomass. Biomass accumulation in soybean is essential for allocating grain production and filling [49]. When glyphosate was applied at V4, the soybeans captured enough resources to maintain crop growth rate during the reproductive stage to achieve high yields [50]. However, the application at R1 was not as effective as the application at V4 in reducing weed competition. Therefore, crop biomass accumulation was lower, as well as yield. Weed presence was enough to cause competition with soybean and therefore limit crop biomass production. The application at R1 may have been beyond the critical timing for weed removal, although this is highly variable between years and locations, and depends on several crop management factors [51]. For soybean, this period is around V2–V4 [21,52–54].

As most individuals in weed populations belong to the first cohort, they are similar in size and likely have similar potential for acquiring resources [55]. Therefore, estimating crop yield loss according to weed density could also be useful for estimating weed crop

competition. The soybean yield in the absence of weeds was  $2.9 \text{ tn ha}^{-1}$ , and the yield losses estimated with the model of the rectangular hyperbola had an adjustment of 0.71, reaching a minimum yield loss of 18% and a maximum yield loss of 82%. The  $I$  value was lower than those obtained for the same species in snap beans [56] and bell pepper [57], and much lower than that reported in sweet potato (61%) [58]. This is likely due to the competitiveness of soybean crop compared to other crops. However, the  $A$  value was higher than those reported in snap beans, bell pepper [56,57], and sweet potato [58].

Glyphosate application at the V4 stage is the optimal timing for short-term weed management due to the reduction of weed survival and prevention of yield loss. It is also the best timing for mid-term management because it lowers fecundity, which, together with the reduced number of reproductive plants, reduces the seeds entering the seedbank. A single glyphosate application in the range of 14 to 28 days after soybean emergence provided effective weed control [59]. However, this technique is more likely to be useful in narrow-row soybeans under favourable growth conditions. In wide-row crops, application timing is more critical and may need a second glyphosate application to control late-emerging weeds [46], or the use of residual herbicides. However, those late-emerging weeds could be harmless to the yield and contribute to the maintenance of a diverse weed community. In the long term, a more diverse weed community could be less competitive to crops and indicate agronomic and environmental sustainability [60,61].

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