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GROWTH, DEVELOPMENT AND SEED PRODUCTION OF GOOSEGRASS[1]

Crescimento, Desenvolvimento e Produção de Sementes de Capim-Pé-de-Galinha

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ABSTRACT - *E. indica* is one of the most problematic weeds in the world because it is present in almost every continent, and there are reports of multiple resistance to herbicides by some biotypes. *The* objective of this paper was to analyze the growth, the development and the production of this plant's seeds, in order to generate information about its biology that can be useful for management. The experiment was carried out in a greenhouse from May to September 2015. Sixteen samples were taken during the development cycle of the plant: 3, 10, 17, 24, 31, 38, 45, 52, 59, 66, 73, 80, 87, 94, 101 and 108 days after emergence (DAE). The response variables were based on a leaf area and dry matter of each one of the parts of the plant and the number of seeds produced per plant. At 12 DAE, 80% of the seedlings of *E. indica* had emerged, and each plant produced more than 120 thousand seeds, closing their cycle at 120 DAE. Between 38 and 43 DAE, the plant had fast emission of new tillers, exponential accumulation of the total dry matter and substantial increase of the absolute growth rate. Due to the data observed here, we concluded that the management of *E. indica* must be done preferably before 38 DAE due to the exponential growth after this period, preventing the plant from producing seeds and spreading to other places.

Keywords: *Eleusine indica, relative growth, biomass allocation, weed biology.*

*RESUMO - **E. indica** é uma das plantas daninhas mais problemáticas do mundo por estar presente em quase todos os continentes, e há relatos da resistência múltipla a herbicidas que alguns biótipos apresentam. O objetivo deste trabalho foi analisar o crescimento, o desenvolvimento e a produção de sementes desta planta, visando gerar informações sobre sua biologia que podem ser úteis para o seu manejo. O experimento foi conduzido em casa de vegetação, durante maio a setembro de 2015. Dezesseis amostragens foram realizadas durante o ciclo de desenvolvimento da planta: 3, 10, 17, 24, 31, 38, 45, 52, 59, 66, 73, 80, 87, 94, 101 e 108 dias após a emergência (DAE). As variáveis-resposta foram baseadas em área foliar e matéria seca de cada uma das partes da planta e no número de sementes produzidas por planta. Aos 12 DAE, 80% das plântulas de **E. indica** haviam emergido, e cada planta produziu mais de 120 mil sementes, encerrando seu ciclo aos 120 DAE. Entre 38 e 43 DAE, a planta apresentou rápida emissão de novos perfilhos, acúmulo exponencial de massa seca total e aumento substancial da taxa de crescimento absoluto. Em função dos dados aqui observados, concluiu-se que o manejo de **E. indica** deve ser feito preferencialmente antes de 38 DAE devido ao crescimento exponencial após esse período, impedindo que a planta produza sementes e se dissemine para outros locais.*

Palavras-chave: *Eleusine indica, taxa de crescimento relativo, alocação de biomassa, biologia de plantas daninhas.*

INTRODUCTION

Eleusine indica, commonly known in Brazil as goosegrass, is considered one of the five most problematic weeds in the world, infesting annual and perennial crops, vegetables and roadsides; it is found mainly in Africa, in America and in intertropical regions of Asia (Holm et al., 1977; Ismail et al., 2002; Mueller et al., 2011). It is a diploid, annual, autogamous species with a C4 photosynthetic mechanism and can produce a high number of seeds (Chauhan & Jhonson, 2008; Jalaludin et al., 2010).

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Several cases of resistance to herbicides in *E. indica* have happened worldwide. Until now, there are reports of biotypes resistant to mitotic inhibitors, ALS inhibitors, ACCase inhibitors, photosystem I inhibitors and EPSPs inhibitors. In Malaysia, there are cases of multiple resistance in which the populations present resistance to EPSPs inhibitors and ACCase, or to photosystem I inhibitors and GS-GOGAT inhibitors, or even to these four action mechanisms simultaneously (Jalaludin et al., 2014; Heap, 2016). In Brazil, failure in the control of goosegrass in the field has been reported frequently due to resistance to the ACCase inhibitors (Heap, 2016) and the low level of resistance to glyphosate (Vargas et al., 2013).

One of the main tools for the management of resistance to herbicides is the implementation of integrated measures in the control of weeds. However, to enable the use of non-chemical control strategies, it is crucial to know the main aspects related to the biology of these plants (Van Acker, 2008). In this sense, studies on the analysis of weeds growth have been done for some species that have some type of resistance to herbicides (Carvalho et al., 2005; Machado et al., 2006).

The analysis of the plants' growth is a highly employed analytic tool to characterize the development, based on data from the dry matter and leaf area resulting from the amount of accumulated biomass in the different organs throughout the cycle (Poorter et al., 2012). Usually, the plants that present faster growth and greater size are those which are more enabled to compete for resources of the environment (Roush & Radosevich, 1985). This type of detailed analysis of a weed also enables the planning of instances for the application of herbicides in phases where they are more susceptible or when the translocation potential of one of the products is higher.

Considering that *E. indica* is a global problem and that the difficulty to control it has increased mainly due to the resistance of herbicides, the objectives of this paper was to analyze growth and development and quantify the production of goosegrass seeds, in order to contribute to the management if this species.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse, located in the city of Maringá - PR, from May to September 2015.

Initially, seeds of Goosegrass were collected from the agricultural area of Maringá (23^{o}20'55.93" S; 52^{o}04'13.76" W), and ripe seeds of at least 10 random plants were removed, being stored in paper bags. After that, a sample representing the entire population was botanized and deposited at the Herbarium of the State University of Maringá (HUEM), later on being identified as *Eleusine indica*.

Right after harvesting, one seed of Goosegrass was put to germinate in each cell of 0.70 mm thick plastic trays, filled with coconut fiber substrate inside the germination chamber (BOD) with daily configuration of eight hours of light at 35 ^{o}C and 16 hours without light at 20 ^{o}C – conditions considered optimal for germination (Ismail et al., 2002). The number of plants obtained in this phase was above the necessary, because not all seeds had uniform germination. Therefore, uniform plants were selected for the transplantation of a single plant per vase, which was done 14 days after sowing (DAS), when the seedlings had real leaf.

The experimental units were composed of vases with capacity for 3.5 dm^3, filled with soil that presented the following characteristics: pH in water of 6.30; 3.68 $cmol_c$ of $H^+ + Al^{+3}$ dm^{-3} of soil; 3,17 $cmol_c$ dm^{-3} of Ca^{+2}; 0,67 $cmol_c$ dm^{-3} of Mg^{+2}; 0,61 $cmol_c$ dm^{-3} of K^+; 47,60 mg dm^{-3} of P; 11,89 g dm^{-3} of C; 640 g kg^{-1} of coarse sand; 50 g kg^{-1} of thin sand; 20 g kg^{-1} of silt; 290 g kg^{-1} of clay; and sandy loam-clay texture. Before transplantation, the soil was fertilized with the equivalent to 200 kg ha^{-1} of formulated fertilizer (10-10-10). During the experiment, irrigation depth equivalent to 7 mm $day^{-1}$was applied.

The plant harvests were done weekly in 16 dates during the development cycle: 3, 10, 17, 24, 31, 38, 45, 52, 59, 66, 73, 80, 87, 94, 101 and 108 days after emergence (DAE). The date of emergence was considered the day in which approximately 80% of the seedlings emerged completely on the trays with substrate at 12 DAS (two days before transplantation). The experimental design was entirely randomized, with four replications. In each evaluation, four plants, or replications, were randomly evaluated destructively. Each plant was carefully removed from the vase and washed in running water to remove the remaining soil from the roots.

The variables initially analyzed were: leaf area (LA), through a leaf area meter model LICOR LI-3100 (LI-COR, inc., Lincoln, Nebraska, USA), and stage of development, through a phenological scale for BBCH weeds (Hess et al., 1997). After that, the material sampled was dried in a greenhouse at 65 ^oC for 72 hours so that, after drying, the leaf dry matter (LDM), the culm dry matter (CDM), the roots dry matter (RDM) and the total dry matter (TDM) of the plants were determined.

The inflorescence dry matter (IDM) was also evaluated and floral racemes in each one of the evaluated plants were counted, as well as the average length of these structures from the first evaluation after the emission of the first inflorescence (45 DAE). In each of the evaluation dates, in order to estimate the total number of seeds produced per plant, the number of seeds in 10 mm of raceme were counted, randomly, in 100 samples, as proposed by Carvalho et al. (2005). Therefore, it was possible to estimate the amount of seeds produced by the product between the number of seeds in 10 mm of raceme, the average length of racemes and the number of racemes per plant.

In each evaluation time, with the total dry matter values (TDM), it was possible to calculate the absolute growth rate (AGR) in g day^{-1}, with the formula: AGR = $(TDM_n - TDM_{n-1})/(t_n - t_{n-1})$, in which TDM_n and TDM_{n-1} are the total dry matter of two consecutive samples and $(t_n - t_{n-1})$ are the days elapsed between these two evaluations. There were

Table 1 **-** Stage of development of plants of *Eleusine indica* in function of the evaluation period (in days after sowing – DAS and days after emergence – DAE) and characterization through the BBCH scales (Hess et al., 1997). Maringá-PR/2015

Date	DAS	DAE	Development Stage	
			Characterization*	BBCH Scale
28/May	0	-	Sowing	0
09/June	12	0	Emergence	9
12/June	14	2	1-2 leaves	12
16/June	19	7	3 leaves	13
18/June	21	9	4 leaves, tillering	22
22/June	25	13	4-5 leaves, tillered	22
24/June	27	15	4-5 leaves, tillered	23
30/June	33	21	5-6 leaves, tillered	24
03/July	36	24	6-7 leaves, tillered, booting	25
08/July	41	29	6-7 leaves, tillered, tassel emission	27
17/July	50	38	6-7 leaves, tillered, tasseled	59
22/July	55	43	7 leaves, tillered, tasseled	61
24/July	57	45	7 leaves, tillered, tasseled	65
31/July	64	52	7 leaves, tillered, tasseled	67
07/August	71	59	7 leaves, tillered, tasseled	69
14/August	78	66	7 leaves, tillered, tasseled	71
21/August	85	73	7 leaves, tillered, tasseled	73
28/August	91	80	7 leaves, tillered, tasseled	75
04/September	99	87	7 leaves, tillered, tasseled	77
11/September	106	94	7 leaves, tillered, tasseled	79
18/September	113	101	7 leaves, tillered, tasseled	81
25/September	120	108	7 leaves, tillered, tasseled	90

* Number of leaves referring to the main tiller.

also calculations of the relative growth rate (RGR), which expresses the growth of a plant in a timeframe, considering the dry biomass accumulated in the beginning of this interval, calculated by the formula: RGR = $(\ln TDM_n \ \ln TDM_{n\ 1})/(t_n \ t_{n\ 1})$.

Finally, the net assimilation rate (NAR) was calculated, representing the net photosynthesis rate and being determined by the relation between the dry biomass produced by the plant's leaf area in a certain timeframe, calculated by the formula: NAR = $[(TDM_n\text{-}TDM_{n\text{-}1})/(t_n\text{-}t_{n\text{-}1})]. [(\ln LA_n\text{-} \ln LA_{n\text{-}1})/LA_n\text{-} LA_{n\text{-}1})]$, in which LA_n is the leaf area of the plant on the occasion of evaluation n; and $LA_{n\text{-}1}$ is the leaf area of the plant on the occasion of evaluation n^{-1}. All calculations and formulas used in these variables were followed according to Portes & Castro Jr. (1991), Poorter & Garnier (2007) and Hoffmann & Poorter (2002).

The quantitative variables related to the growth of the plant were analyzed through the variance analysis and compared by the F test ($p<0.05$). For the variances that present significance, linear and non-linear regression models were adjusted, taking into account the significance of the estimated coefficients and also the biological explanation for the phenomenon. For the non-linear models, the Logistic (1) and the Lorentzian (2) models were adopted:

$$y=a+\frac{b}{\left[1+\left(\frac{x}{c}\right)d\right]} \qquad \text{(eq. 1)}$$

$$y=a+\frac{b}{\left[1+\left(\frac{x-c}{d}\right)^2\right]} \qquad \text{(eq. 2)}$$

The equation's parameters are represented by *y*, which is the response variable; *x* is the amount of accumulated days; and *a*, *b*, *c* and *d* are the adjustment parameters of the equation, so that *a* is the minimum point obtained, *b* is the difference between the maximum and minimum point, *c* is the amount of days that provides 50% of the response variable for model (1) and 100% of the variable response for model (2), and *d* is the curve steepness.

RESULTS AND DISCUSSION

The seeds of *E. indica* presented emergence above 80% in the daily conditions of temperature and lighting to which they were subjected (8 hours of light at 35 °C and 16 hours without light at 20 °C). Papers in the literature show that thermal and light fluctuation are determining factors for the seeds of *E. indica*, seeing that its germination is below 10% under constant temperatures between 20 and 35 °C, while it is increased to 99% under alternate temperature conditions at 16 hours at 20 °C and 8 hours at 35 °C (Nishimoto & MacCarty, 1997; Ismail et al., 2002).

Something common and intrinsic to the weeds is the ability to keep their germination power for a long period after dispersion of their seeds. In this paper, the emergence of 80% of the seeds of *E. indica* happened along 12 days, which is a relatively bigger timeframe compared to the usually required time for emergence in annual crops, such as soybeans and corn, which have an average time of 5 to 7 DAS, respectively (Viana et al., 2005; Schuab et al., 2006). This means that, in a situation of initial competition between these crops and the Goosegrass in an area where the plants have not emerged yet, these crops will possibly emerge before this weed.

Initially, the accumulation of total dry matter of *E. indica* happened slowly until 38 DAE (Figure 1). From that moment on, there was an increase, following the exponential trend of this variable to the curve inflection point, at 53 DAE. The curve inflection point indicates that the plant stops having exponential growth and begins to accumulate less dry matter throughout time. For the leaves, culms and roots, the accumulation of dry matter throughout the development cycle of the plant occurred similarly. Usually, the species that usually have a faster growth are abler to compete for resources of the medium (Roush & Radosevich, 1985). With that, we understand that, from 38 DAE on, *E. indica* 's ability to compete also increases, but it is worth mentioning that, in an interspecific

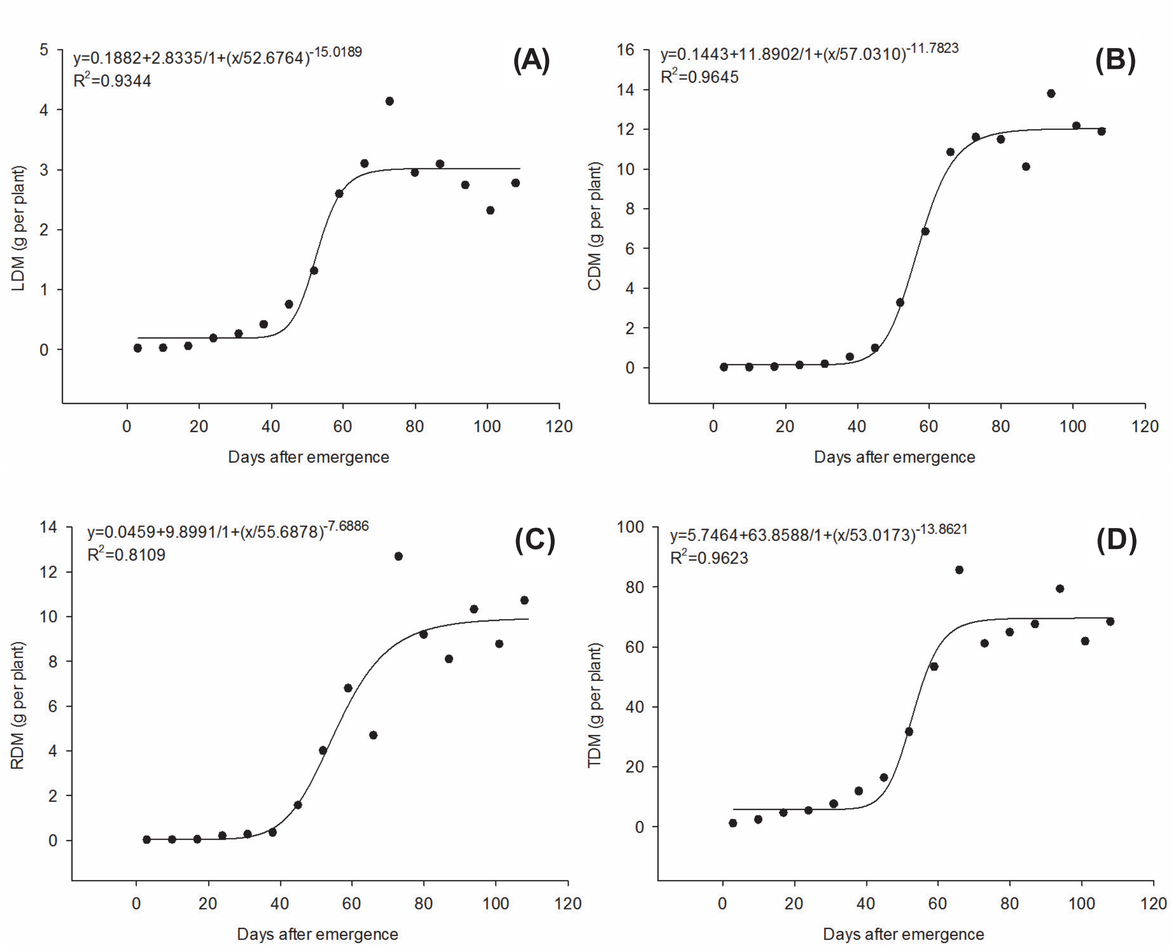

Figure 1 - Leaves Dry Matter – LDM (A), culms dry matter – CDM (B), roots dry matter – RDM (C) and total dry matter – TDM (D) throughout the cycle of goosegrass development. Maringá – PR/2015.

coexistence condition, this period may be different.

The beginning of tillering happened after the emission of the third leaf (9 DAE), but tillering happened slowly until 38 DAE. From that moment on, there was intense tillering that extended until the curve inflection point, at 53 DAE. The tillering number per plant was established only at 71 DAE, simultaneously to the accumulation of TDM (Figure 2). At 76 DAE it was also possible to observe the maximum leaf area that until that point presented an exponential increase and that, from that moment on, is reduced drastically.

When comparing the growth of *E. indica* to that of the other monocot weeds, similar behaviors were observed for *Digitaria insularis* and for *Chloris polydactyla*, which present slow dry matter accumulation until around 45 DAE. *C. polydactyla* begins its tillering at 30 DAE, and the beginning of flowering is at 106 DAE. In turn, for *D. insularis,* flowering happens at 70 DAE (Carvalho et al., 2005; Machado et al., 2006). Based on these factors, *E. indica* has a faster development cycle than other species, seeing that it is an annual cycle species, with more adaptability in environments with high level of disturbance, due to its capacity of generating new individuals early on (Poorter & Gainer, 2007).

Throughout the plant development cycle, variations were observed in the partition of the biomass for the leaves, the culms, the roots and the inflorescences (Figure 3). Initially, 60% of the total dry matter of the plant was composed of leaves and the remaining 40% of roots. At 38 DAE, the allocation of biomass

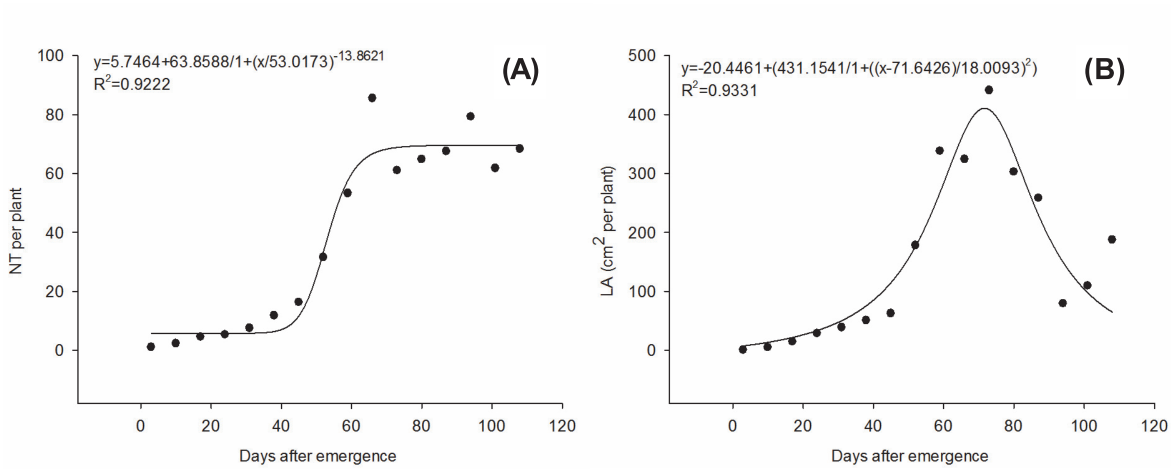

Figure 2 **-** Number of tillers – NT (A) and leaf area – LA (B) throughout the development cycle of goosegrass. Maringá – PR/2015.

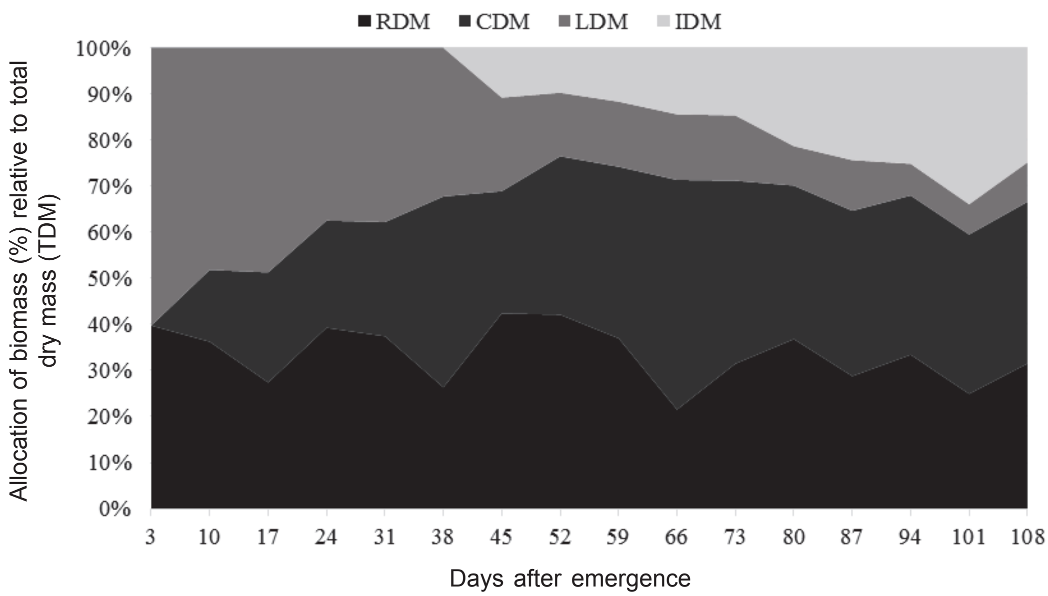

Figure 3 **-** Allocation of biomass in different parts of the plant (%) throughout the development cycle of goosegrass. Leaves Dry Matter – LDM, culm dry matter – CDM, roots dry matter – RDM and inflorescences dry matter – IDM. Maringá – PR/2015.

between leaves, culms and roots was 33, 40 and 27%, respectively. From the beginning of the inflorescences emission, at 29 DAE, one can observe that the participation of leaves dry matter in the total dry matter of the plant was reduced, due to the reduction of its leaf area, original from the drain of photoassimilates for the production of seeds.

The quadratic regression model was adjusted for the relative growth rate (RGR) throughout the days of the development cycle of Goosegrass (Figure 4). This happens because this variable represents an accumulation of dry matter throughout time, considering the pre-existing biomass in the plant, differently than the absolute growth rate (AGR) (Poorter et al., 2012). In addition, with the increase of dry matter accumulated by the plants, there is greater need of the photoassimilates to maintain the already formed structures, decreasing the amount of energy available for its growth (Benincasa, 2003).

Maximum RGR happened at 10 DAE, decreasing as the days went by, until it reached negative values in the end of the plant's cycle. In turn, the AGR had a substantial growth from 43 DAE, simultaneously with the exponential increase of the accumulation of the total biomass of the plant. The maximum values of RGR and AGR were 0.15 g g^{-1} day^{-1} and 0.82 g day^{-1}, respectively. These values were similar to the growth rates of other species of C4 weeds, such as *D. insularis* (Machado et al., 2006) and *Cyperus rotundus* (Brighenti et al., 1997), which have a maximum RGR ranging from 0.12 to 0.14 g g^{-1} day^{-1}.

With the decrease of the leaf area due to the photoassimilates drain for the formation of seeds, the net assimilation rate also decreased due to the limitation of the main responsible structures for the production of photoassimilates (leaves) (Figure 5). In these situations, the plant tends to allocate biomass to leaves, culms and roots in a balanced way, in order to meet the physiologic needs and the functions performed by these organs (Poorter et al., 2012).

In this paper, the beginning of seeds production of Goosegrass happened at 38 DAE – timeframe below the cycle of many annual crops. The number of seeds per plant increased continuously until the end of the evaluation period, which indicates that the species produces a growing number of seeds through a period of at least 70 days (38 to 108 DAE). The continuous production of seeds implies in the unevenness of its maturation, which can also contribute to a survival strategy. In crops of

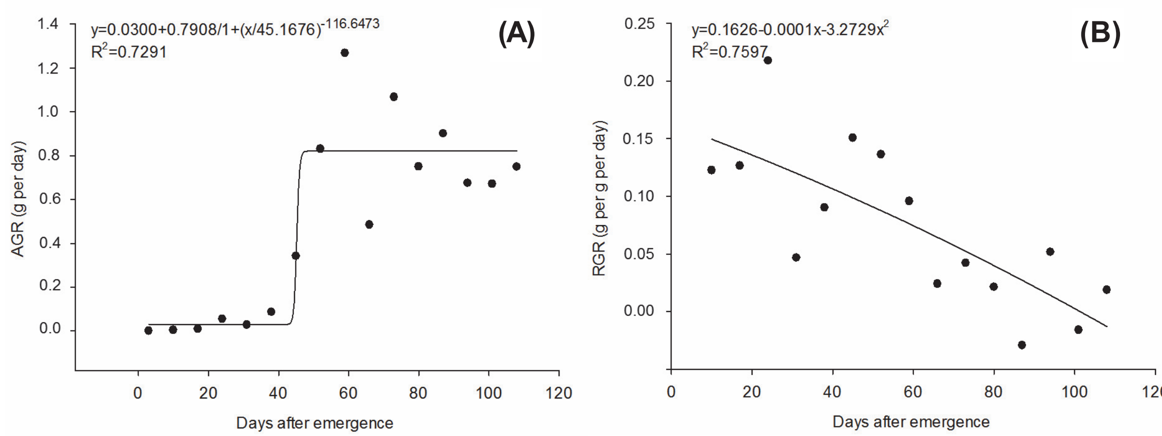

Figure 4 **-** Absolute growth rate – AGR (A) and relative growth rate – RGR (B) throughout the development cycle of goosegrass. Maringá – PR/2015.

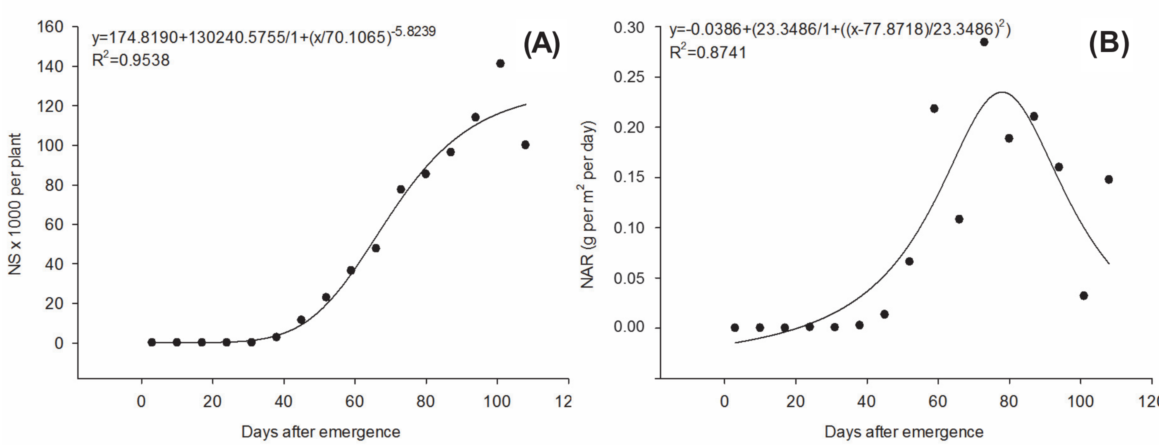

Figure 5 **-** Number of seeds per plant – NS (A) and net assimilation rate – NAR (B) throughout the development cycle of goosegrass. Maringá – PR/2015.

grains, in case the plant control of *E. indica* is ineffective, they will be able to produce seeds before the end of the crops cycle, which means they can be able to disperse their propagules through the grain harvester, powering their capacity of infesting new areas (Walsh & Powles, 2014). The maximum value of a number of seeds happened at 108 DAE, when this species was able to produce more than 120 thousand seeds per plant (Figure 5). In that sense, practices that prevent the production of seeds must be considered one of the key points for a sustainable management of the seeds bank of *E. indica* in the crops (Chauhan & Johnson, 2010).

Generally, the crops have less competitive ability than the weeds, but, in most cases, the high density of weeds in the area has strong influence on the interference degree (Bianchi et al., 2006). However, studies of competition between Goosegrass and crops such as soybeans showed similar competitive abilities between the weeds and the crops (Wandscheer at al., 2013).

The cycle of *E. indica* was considered ended at 120 DAE, when the plant initiated the natural senescence. In competitive conditions, different results may be observed because their physiologic characteristics are usually altered, resulting in differences in the use of environment resources, especially in the use of water, which influences directly on CO_2 availability, on the temperature of the leaf and, consequently, on the plant's photosynthetic efficiency (Concenço et al., 2007).

Considering that from 38 DAE there is exponential growth in the accumulation of dry matter, as well as high values of AGR from 43 DAE, in coexistence of *E. indica* with cultivated species in situations in which it presents stage 59 BBCH or above, higher losses with interference can be observed. In this sense, it is important that the management of *E. indica* is carried out before the plant reaches this stage. Because the Goosegrass is a species that has a C4 photosynthetic mechanism, it makes its growth and development even more accelerated in conditions of higher temperatures.

Once in many situations the main control option is chemistry, the early management of *E. indica* should be adopted to avoid initial weed interference with the crops. The control efficiency of Goosegrass with the use of herbicides (glyphosate or ACCase inhibitors) applied in post emergence is inversely proportional to the development stage, seeing better levels of efficacy with applications done in stages of up to four leaves (Ulguim et al., 2013). In this paper, the stage of four leaves was reached at 9 DAE, which indicates that, in order to maximize the chance of success of these applications in post emergence, the applications should be done before or at most when the plants of *E. indica* have reached the stage 22 BBCH.

Considering the results obtained, we conclude that, with a regime of 8 hours of light at 35 °C and 16 hours in the absence of light at 20 °C daily, *E. indica* needed 12 days to emerge in 80%. Tillering starts at 9 DAE, and the seed production at 38 DAE, being able to produce more than 120 thousand seeds per plant at 108 DAE and finishing the cycle at 120 DAE. Between 38 and 43 DAE, the plant presents fast emission of new tillers, exponential accumulation of the total dry matter and substantial increase of the absolute growth rate. Therefore, control measures for *E. indica* must be adopted, especially before 9 DAE. After this period, the control of herbicides is smaller (Ulguim et al., 2013), just like before the 38 DAE, when the exponential growth phase and the production of seeds starts, which potentiates the capacity of competition and dissemination of propagules.

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