PLANTA DANINHA
SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS
<http://www.sbcpd.org>
ISSN 0100-8358 (print)
1806-9681 (online)

Article

LONGEVITY OF HORSEWEED SEED BANK DEPENDING ON THE BURIAL DEPTH

Longevidade do Banco de Sementes de Buva em Função da Profundidade de Enterrio

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ABSTRACT - Horseweed (*Conyza* spp.) is considered as one of the major weeds occurring in the agricultural areas of South America, especially in Brazil. This species stands out from the other weeds due to characteristics such as large seed production, continued seed production, dispersion over long distances and resistance to 5-enolpyruvoylshikimate 3-phosphate (EPSP) and acetolactate synthase (ALS) inhibiting herbicides, features that increase their management. Therefore, this study aimed at determining the physiological quality and longevity of horseweed seeds over 12 months. The experiment was established under field conditions, using a randomized block design with four replications. Fifty horseweed seeds were distributed into 50 g of dry soil, placed in permeable nylon mesh bags (10 x 10 cm), which were buried at 1, 2, 4 and 8 cm of depth. Each month, for 12 months, samples were collected and washed with water, and the remaining seeds were submitted to germination test. The percentage (%) of remaining seeds, germination, abnormal seedlings, dead seeds, dormancy and viability were evaluated. It was found that the horseweed seed bank was reduced by 59 % over 12 months. Moreover, horseweed seeds develop a secondary dormancy, regardless of the burial depth. In addition, an increased burial depth increases longevity and physiological seed quality.

Keywords: *Conyza* spp., survival, dormancy, germination, viability.

*RESUMO - A buva (**Conyza** spp.) é considerada uma das principais plantas daninhas que ocorrem em áreas agrícolas da América do Sul, especialmente no Brasil. A espécie destaca-se entre as demais plantas daninhas devido a características como elevada produção de sementes, produção continuada, dispersão a longas distâncias e também por apresentar resistência a herbicidas inibidores da 5-enolpiruvilchiquimato-3-fosfato sintase (EPSP) e acetolactato sintase (ALS), o que dificulta seu manejo. Assim, o objetivo deste trabalho foi determinar a qualidade fisiológica e a longevidade de sementes de buva no período de 12 meses. O experimento foi conduzido em campo, utilizando delineamento em blocos casualizados com quatro repetições. Foram distribuídas 50 sementes de buva em 50 g de solo seco, alocadas em sacos de malha de náilon permeável (10 x 10 cm), os quais foram enterrados a 1, 2, 4 e 8 cm de profundidade. A cada mês, foram feitas coletas, sendo as amostras lavadas com água corrente e as sementes remanescentes submetidas ao teste de germinação. Foram avaliadas a porcentagem (%) de sementes remanescentes, germinação, plântulas anormais, sementes mortas, dormência e viabilidade. Verificou-se que o banco de sementes de buva foi reduzido em 59% no período dos 12 meses. As sementes sobreviventes de buva desenvolvem dormência secundária, independentemente da profundidade de enterrio. O aumento da profundidade de enterrio eleva a longevidade e preserva a qualidade fisiológica das sementes.*

Palavras-chave: *Conyza* spp., sobrevivência, dormência, germinação, viabilidade.

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Received: December 14, 2016
Approved: March 10, 2017

Planta Daninha 2018; v36:e0182073

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Doi: 10.1590/S0100-83582018360100050

INTRODUCTION

Horseweed is a weed of the Astereceae family, which is normally considered to have an annual cycle (Gibson et al., 2005). In Brazil, the occurrence of the species *Conyza canadensis*, *C. bonarensis* and *C. sumatrensis* (Lazaroto et al., 2008; Sansom et al., 2013) is frequent; they are associated with conservation systems, such as no-tillage and minimum soil cultivation (Main et al., 2006). Thus, in conventional cropping systems, horseweed management is relatively simple, and soil preparation can significantly reduce the population of these plants (Davis et al., 2009). However, in no-tillage systems, soil rotation is replaced by the use of herbicides, which, when used repeatedly and incorrectly, select resistant biotypes. Resistance to glyphosate was observed for the horseweed species *C. bonariensis* (Vargas et al. 2007) and *C. canadensis* (Moreira et al., 2007), as well as multiple resistance to EPSPs and ALS inhibitors for *C. sumatrensis* (Santos et al., 2014).

The biological characteristics of these species, such as the large number of viable seeds, the ability to develop under straw and the dispersion over long distances (Yamashita and Guimarães, 2011), change the number and relative dominance of each species in the agroecosystem, making it harder to manage them (Sansom et al., 2013). Usually, weed seeds germinate when conditions are favorable, in soils with enough resources to support the subsequent growth of the plant and thus achieve the survival of the species (Chauhan et al., 2008). Thus, factors such as light become decisive in positively photoblastic seeds, such as horseweed, which require the incidence of a certain quantity and quality of light to start the germination process (Vidal et al., 2007; Vivian et al., 2008; Yamashita et al., 2016).

Light works as a seed positioning sensor on the ground. Under shading conditions, horseweed seeds located at certain depths do not receive proper light, so they cannot respond to the changes in temperature occurring during the year, or to temperature fluctuations throughout the day (Vidal et al., 2007). Thus, light becomes essential for the germination process, and it may induce or suppress dormancy mechanisms (Baskin and Baskin, 2014).

Dormancy is defined according to the moment in which it occurs; it may be primary or innate, programmed when the seed is still in the mother plant, and it may be secondary or induced, when it develops in seeds that are not dormant or that have broken dormancy, in response to unfavorable environmental conditions for germination. There are also dormancy mechanisms that avoid germination due to physiological (preventing radicle protrusion), morphological (incomplete embryo formation), and physical (impermeability to water) causes, and due to the combination of two factors (morphological and physical/physiological) (Baskin and Baskin, 2014). Moreover, seeds may show quiescence, where there is no dormancy, but seeds do not germinate due to one or more unfavorable environmental conditions (Marcos Filho, 2005).

Even though horseweed seeds do not have dormancy, they can maintain their viability in the soil seed bank for relatively long periods (Constantin et al., 2013). *C. bonariensis* seeds showed 7.5, 9.7 and 1.3% viability at depths of 10, 5 and 0-2 cm, respectively, for a period of three years (Wu et al., 2007). Seeds from weeds allocated on the surface or at lower depths have greater exposure to light and favorable environmental conditions for germination (Yamashita et al., 2016). Thus, it was determined that horseweed seeds exhibit a negative interaction between burial depth and emergence; when they remain at more than 0.5 cm depth, emergence is low and may decrease or be zero (Vidal et al., 2007).

In light of the aforementioned, the objective of this work was to determine the physiological quality and longevity of horseweed seeds (*Conyza* spp.) according to the burial depth.

MATERIAL AND METHODS

The experiment was established on the field between May 2014 and May 2015. Tests to evaluate the physiological quality of the seeds were conducted in a seed analysis laboratory.

Seeds were collected from maturing capitula, in a soybean production area, in the municipality of Capão do Leão, Rio Grande do Sul state (31º49'9,6" South latitude and 52º27'30,7" West longitude). They were taken to the laboratory, where mature capitula and only full seeds were selected (100% of the essential tissues for germination: tegument and embryo), with the help of fine tweezers and light stereoscope. After selection, 50 seeds were mixed in 50 g of soil [Red-Yellow Argissol, with a sandy-loam texture (Embrapa, 2013)] and placed in a bag made of nylon mesh (10 x 10 cm).

The experiment was conducted in a completely randomized design with four replications. The vegetation in the experimental area was removed through weeding; each experimental unit was composed of a 0.25 m^2 area. Treatments were placed in a factorial arrangement, where factor A was composed of burial depths (1, 2, 4 and 8 cm), and factor B represented 13 collection periods (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 months).

The bags were placed in the middle of each experimental unit, at different burial depths, according to factor A; they were covered with the removed soil layer. In order to keep the experimental unit free from weeds, glyphosate (480 g L^{-1}) was applied monthly at the rate of three liters per hectare.

For 12 months, collections were made monthly at each depth. In each evaluation, seeds were extracted from the bag by washing the sample, with the help of a sprinkler in a set of sieves. The sample was screened through a set of 16, 32 and 60 mesh sieves. After washing, the sample was placed to dry on filter paper for 24 hours, and then analyzed under a light stereoscope, to extract the remaining seeds.

They were placed in distilled water for two minutes and then submitted to germination test on blotter paper, which was weighed and placed in gerbox boxes and moistened with distilled water at three times its weight. The analysis was conducted in a germination chamber, at a continuous temperature of 20 °C and a 24 hours light photoperiod (Vidal et al., 2007).

The following variables were evaluated: remaining seeds; germination at first and second count, 7 and 14 days after sowing (DAS) (Vivian et al., 2008); abnormal seedlings; dead seeds; and dormant seeds at 14 DAS. The percentage of remaining seeds was calculated based on the initial number of seeds per replication, while for germination percentages, abnormal seedlings, dead and dormant seeds, were estimated based on the number of remaining seeds.

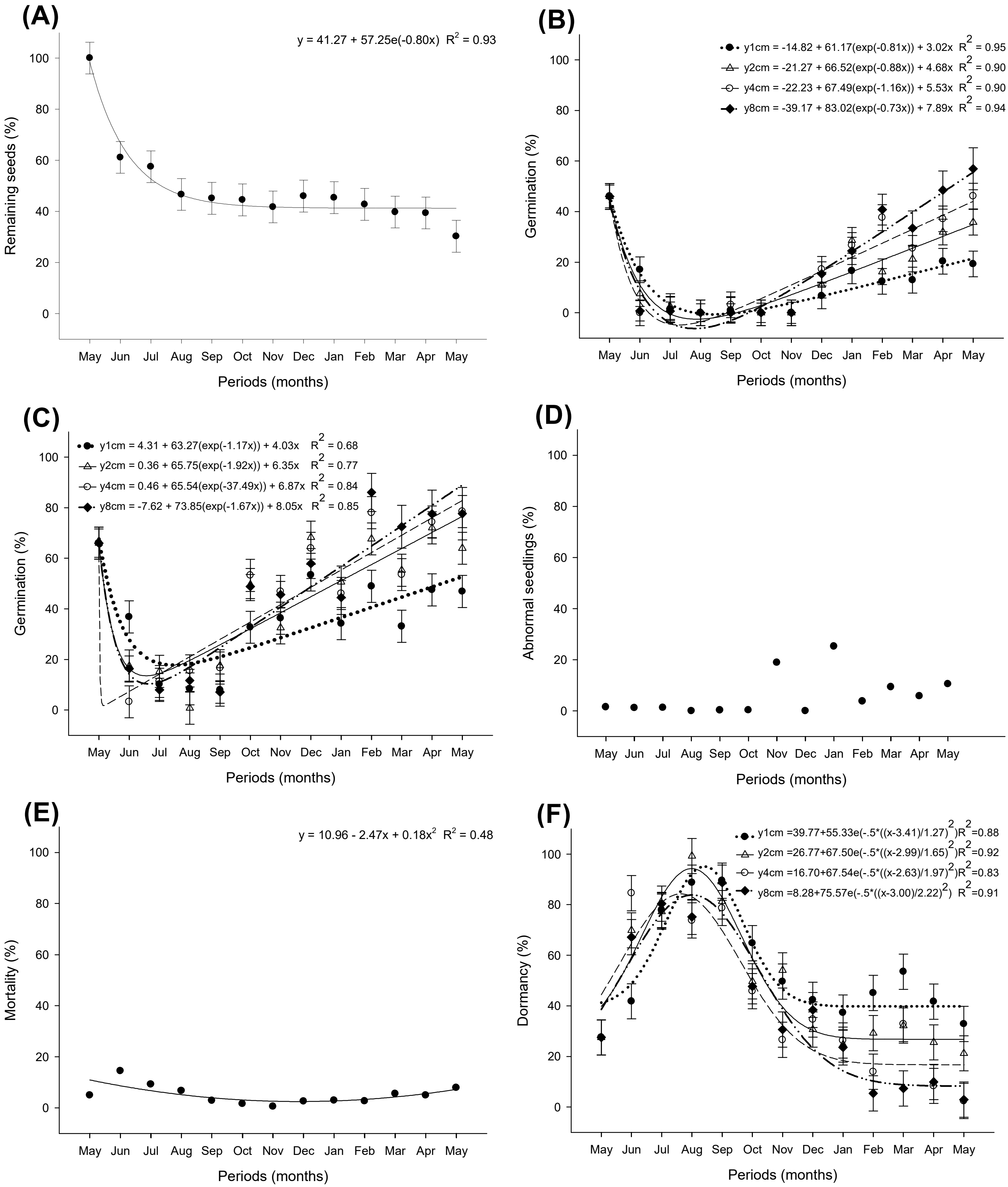
Once the germination test was finished, the viability of dormant seeds was tested through the tetrazolium test with the 2, 3, 5, triphenyl chloride tetrazolium salt at 1.0%, in which it was possible to consider as viable seeds those showing pink or carmine color on the whole seed (Brasil, 2009). For this, seeds were placed in the tetrazolium solution in a transparent glass container and closed with aluminum foil, to prevent the passage of light, for 24 hours at a temperature of ± 30 °C (Brasil, 2009). The percentages of viable and non-viable seeds were calculated based on the number of dormant seeds observed in the germination test.

Data were analyzed as for normality and, subsequently, they were submitted to analysis of variance ($p \leq 0.05$). In case of statistical significance, a regression analysis was performed for the collection period factor, using the SigmaPlot 10.0 program (Sigmaplot, 2007); for the burial depth factor, means were compared by Duncan test ($p \leq 0.05$). Data on abnormal seedlings and dead seeds were submitted to quadratic root type transformation. Moreover, the time variation (months) of the seed status in the soil, according to each burial depth, was calculated from the means for the variables: germination, abnormal seedlings, and seeds (dead, viable, non-viable and predated or deteriorated).

RESULTS AND DISCUSSION

The analysis of data showed a significant interaction between the studied factors as for the first and second germination count, and dormant and viable seed variables. For the remaining and non-viable seed variables, a simple effect of the factors was observed; and for abnormal seedlings and dead seeds, a simple effect of the collection period factor was observed.

As for the remaining seed variable, there was a decrease, followed by stabilization in the percentage of remaining seeds, as the collection period increased; data fitted the decreasing exponential regression equation (Figure 1A). Thus, comparing the first and the last evaluation period, at the average of the four burial depths, it was possible to observe a 59% of reduction in



The dots represent the mean values of the replications, and the vertical bars represent the respective confidence intervals of the mean, with 95% significance.

Figure 1 - Remaining seeds (A), first (B) and second (C) germination count, abnormal seedlings (D), mortality (E) and dormancy (F) in horseweed (*Conyza* spp.) seeds, after the germination test sowing, according to the burial depth and the collection period.

the amount of remaining seeds. This decrease occurred until August (fourth collection period), after that it remained constant, at values close to 40%. The seed bank in the soil can undergo dramatic changes, modifying its dynamics and the proportion and quantity of seeds (Main et al., 2006). Seed bank losses are due to germination, migration and degradation/predation by microorganisms and invertebrates (Chee-Sanford et al., 2006). Moreover, changes in soil temperature can cause the germination or deteriorate more rapidly the seeds (Main et al., 2006). In a study with *C. canadensis*, seed survival was observed for periods from one to three years (Wu et al., 2007).

When depths were tested, was observed a lower percentage of seeds remaining at the depth of 1 cm, and no differences were observed between the depths of 2, 4 and 8 cm (Table 1). Similar results were obtained by Wu et al. (2007), where the increase of the burial depth increased the conservation of *C. canadensis* seeds, thus demonstrating a positive interaction between seed depth and its survival. Moreover, at lower burial depths, seeds have greater light incidence, which could promote germination (Yamashita et al., 2016). This explains the high incidence and adaptability of this weed to no-tillage systems, in which there is no soil rotation, facilitating its emergence and conservation in the system (Vidal et al., 2007).

As for the first germination count, data fitted to the decreased exponential regression equation (Figure 1B). Values were reduced in relation to the initial germination, remaining constant from June (second period) to November (seventh period), and from December (eighth period) on, there were increases at each collection. A study revealed the occurrence of a primary dormancy as a physiological mechanism in horseweed seeds (Karlsson and Milberg, 2007), although there are studies that indicate the absence of dormancy in seeds from this species (Lazaroto et al., 2008; Constantin et al., 2013). In this study, it was possible to observe initial germination and reduction after the first collection period; this can be considered as a secondary dormancy. In case of species such as horseweed, which show primary dormancy as physiological mechanism (Karlsson and Milberg, 2007), with unfavorable environmental conditions for germination, they have the capacity to develop a secondary dormancy (Baskin and Baskin, 2014).

Table 1 - Remaining horseweed seeds (*Conyza* spp.), according to the burial depth

Depth (cm)	Remaining seeds (%)	Non-viable seeds (%)
1	39 b	75 a
2	49 a	67 a
4	53 a	52 b
8	56 a	33 c
VC (%)	40	39

Means followed by the same lowercase letter in the column do not differ by Duncan's test ($p \leq 0{,}05$).

Starting from February (tenth period), the increment of the burial depth increased the germination of the seeds (Figure 1B). These results are due to the fact that the greater depth maintains environmental conditions more constant and, as a consequence, seeds have greater vigor. Weed seeds may reach secondary dormancy when the environmental conditions are not favorable for germination (Vivian et al., 2008). This is an adaptation to prevent germination under unfavorable conditions.

Data showed a higher germination percentage at 8 and 4 cm, followed by 2 cm, which was not different from 4 cm, with lower values at the depth of 1 cm (Figure 1B). The germination increase of the seeds at a greater burial depth suggests more physiological quality for them at these depths. Seeds that remain on the soil surface or at depths closer to the surface are more easily exposed to deterioration/predation and environmental changes, which consequently affect seed quality, in terms of physiological properties such as vigor and germination, as verified in this study.

Analyzing the germination 14 DAS, were found similar results to those exhibited at seven DAS (Figure 1C). There was a decrease in the germination percentage, which was constant from June (second period) to September (fifth period); from October on (sixth period), there were constant increases, and data fitted the decreased exponential regression equation. It is reported that dormancy is a condition that can be caused by the absence of light (Baskin and Baskin, 2014). In this regard, a study confirmed that horseweed seeds kept in the dark remain quiescent, but when are transferred to light, they complete their germination normally (Yamashita et al., 2016).

There was a germination decrease from June (second period) to September (fitth period); from October on (fifth period), there were increases at all depths, with a trend towards higher values as the burial depth increased (Figure 1C). In May (last period), data report higher germination percentages at 2, 4 and 8 cm, and lower values at 1 cm, which was not different from 2 cm. These results reveal that, regardless of the depth, horseweed seeds also reach secondary dormancy. Thus, horseweed seeds located at certain depths do not receive adequate light and cannot respond to the temperature changes occurring during the year, or to the same fluctuations throughout the day, since light and temperature work as positioning sensors for seeds in the soil (Vidal et al., 2007) and generate physiological and biochemical defense mechanisms to preserve and protect it in the soil for longer (Davis et al., 2008).

As for the abnormal seedlings, the observed values were generally low for all evaluated periods, and data did not fit the tested regression equations (Figure 1D). Likewise, the values for dead seeds were low, and data fitted the quadratic polynomial regression equation; minimum value was obtained in November (eighth period) (Figure 1E).

The physiological quality of the remaining seeds was slightly altered by the tested treatments, demonstrating that seeds under these conditions can maintain their quality, germinate and develop a normal seedling, with high reproductive capacity. Knowing the characteristics of dispersion and adaptability of horseweed, these results confirm the problem of horseweed management in no-tillage systems, in which there is no soil plowing. Seeds can remain buried without this affecting their quality, hence the need to establish management strategies to avoid increases to the horseweed seed bank.

As for the dormant seed variable, data fitted the Gaussian normal distribution regression equation (Figure 1F). The maximum percentages of dormant seeds were reached at 3.4, 3.0, 2.6, and 3.0 months of collection at depths of 1, 2, 4 and 8 cm, respectively. The minimum values of the percentages were 39.8, 26.8, 17.8, and 8.3; for 1, 2, 4 and 8 cm, respectively. At the four tested depths, the maximum percentage of dormant seeds was obtained at similar periods, revealing that only the burial condition causes the seed to remain dormant, as a strategy to increase its longevity (Davis et al., 2008).

From December on (eighth period), the percentages of dormant seeds remained constant at depths of 1 and 2 cm, with greater values at 4 and 8 cm, which stabilized in January and February, respectively (Figure 1F). Seeds of most annual weeds have the ability to remain in the soil seed bank for several years (Davis et al., 2008). For horseweed, this period can be up to three years (Wu et al., 2007). Under these conditions, seeds develop both biochemical and physical protection characteristics (Davis et al., 2008). Thus, biochemically speaking, seeds can concentrate secondary metabolites such as phenols and alkaloids (Veldman et al., 2007), and can physically develop their tegument with greater resistance to mechanical damages (Davis et al., 2008). These developed characteristics protect seeds from attacks by pathogens, and provide greater resistance to unfavorable environmental conditions; this may explain the low results of abnormal and dead seedlings.

Analyzing the viable seed variable, a lower viability loss was observed at the greatest burial depths, and a percentage decrease was observed as the collection period increased, for all four depths; data fitted at the linear regression equation (Figure 2A). Thus, for each collection period, seeds decreased their viability by 6.0, 5.9, 6.6 and 7.5%, at depths of 1, 2, 4 and 8 cm, respectively. Studies confirm a great viability loss in the seed bank of the species *C. bonariensis* (Wu et al., 2007) and *C. canadensis* (Weaver, 2001), as observed in this study.

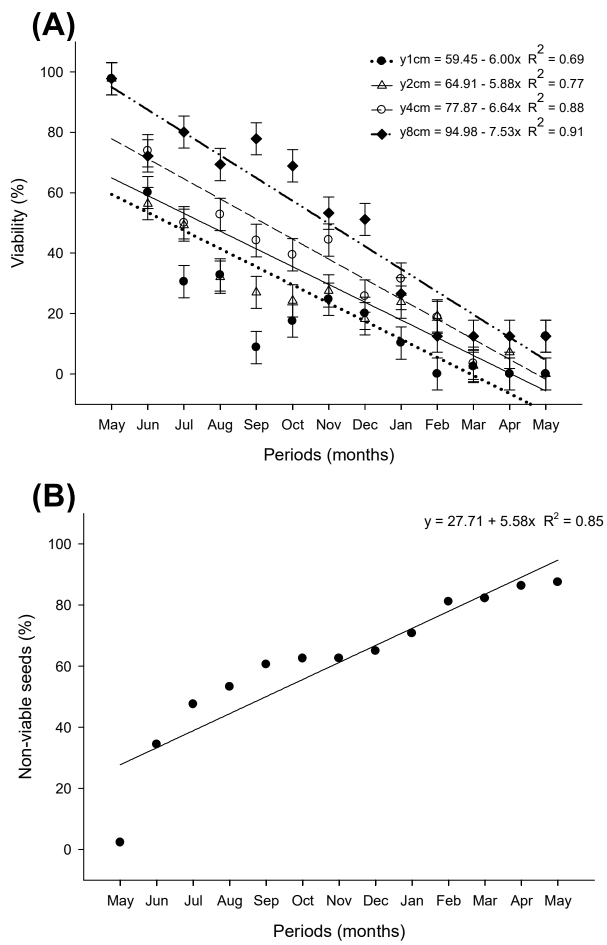
Small seeds, such as horseweed ones, may have lower longevity, due to small embryos (Probert et al., 2009) with few reserves. Although the loss of viability is great during the first year, it then remains constant, observing viable seeds up to two years after the first one (Wu et al., 2007).

Data show a faster viability loss at lower depths; from the ninth month on (ninth period), no differences were observed between the tested depths, with low or null viability values (Figure 2A). Similar results to those in this work were obtained by Wu et al. (2007) when they tested the longevity of *C. bonariensis*; the lowest viability was at depths from 0 to 2 cm, and the highest ones at 5 and 10 cm.

As for non-viable seeds, there were increases of 5.6% in each collection period (month), and data fitted the linear regression equation (Figure 2B). Although seeds are able to remain in the seed bank with their structure unharmed, over time they lose viability, due to the normal process of seed deterioration, which can be faster depending on the environmental conditions (Kapoor et al., 2011).

There was a higher percentage of non-viable seeds at depths of 1 and 2 cm, followed by 4 cm, while 8 cm was the depth with the lowest percentage (Table 1). The longevity of the seeds in the bank depends on the depth at which they are allocated (Davis et al., 2008); however, in a study, there was a greater viability loss from *C. bonariensis* seeds at the lowest depths (Wu et al., 2007), as also observed in this study.

Observing the time variation of the seed status, according to its burial depth, showed a gradient according to depth (Figure 3). At greater depth, seeds showed higher



The dots represent the mean values of the replications, and the vertical barsrepresent the respective confidence intervals of the mean, with 95% significance.

Figure 2 - Viability (A) and non-viable (B) horseweed (*Conyza* spp.) seeds after the germination test sowing, according to the burial depth and the collection period.

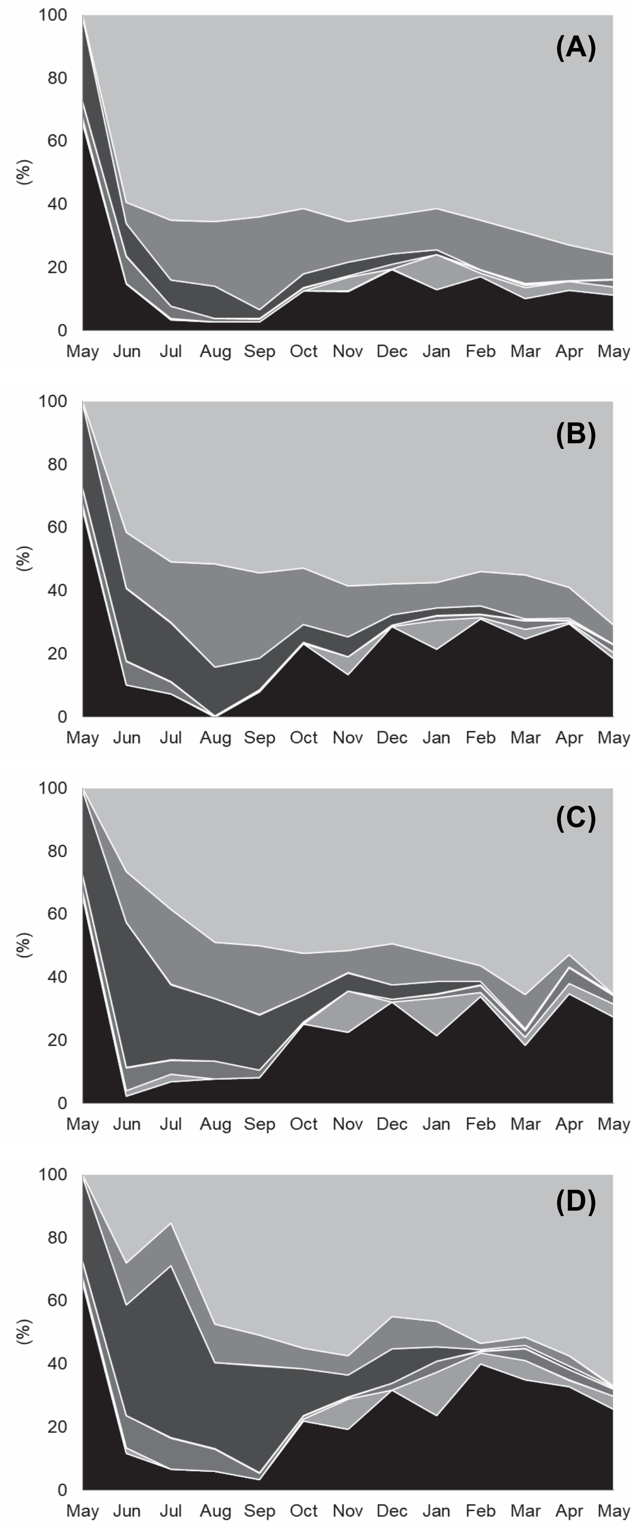


Figure 3 - Variation in time (months) of the status of *Conyza* spp. seeds in the soil, according to the burial depth: 1 (A), 2 (B), 4 (C) and 8 (D) cm; as for germination ■, abnormal seedlings ■, dead ■, viable ■, non-viable ■ and predated or deteriorated ■ seeds.

germination and viable seeds, and less predated or deteriorated seeds. Furthermore, the joint analysis confirms that, at greater burial depths, the quality of horseweed seeds was better.

The results of this study revealed a 59% reduction of the horseweed seed bank in one year. Seeds allocated at greater depth maintain more constant environmental conditions, thus having greater vigor. Furthermore, surviving seeds develop a secondary dormancy, regardless of the burial depth. In addition, an increased burial depth increases the longevity and physiological quality of seeds. This highlights the need for changes in the management of this weed, both in direct seeding systems and in conventional systems, in order to define more appropriate control strategies.

ACKNOWLEDGEMENTS

To the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico - National Council for Scientific and Technological Development), to the University of Costa Rica (UCR), the Ministry of Science, Technology and Telecommunications (MICITT - Ministerio de Ciencia, Tecnología y Telecomunicaciones) and to the National Council for Scientific and Technological Research (CONICIT - Consejo Nacional para Investigaciones Científicas y Tecnológicas) for the financial support.

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