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CROP PROTECTION

Survival and distribution of weedy rice seedbank after twenty-two years of different rice cropping systems

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ABSTRACT: Weedy rice (*Oryza sativa* L.) is the most problematic weed in rice fields due to the few control management alternatives to control it, because of the genetic similarity with the crop. Different cropping systems (regarding soil preparation before sowing) have been used as options to control the persistence and emergence of the weed seedbank. Therefore, the objective of this study was to evaluate the longevity and vertical distribution of weedy rice seeds in the soil seedbank after 22 years of different rice cropping systems. Data was analyzed as a two-way factorial, with cropping systems carried out for 22 years [no-tillage (NT), conventional tillage (CT), wet direct-seeded (WDS)] as one factor and sampling depth (0-2, 2-5, 5-10 and 10-20 cm) as the other factor. The number of whole and deteriorated seeds per m^{-2} were assessed, as well as the viability (%). No effect between the systems were detected up to 5 cm, however at 5-10 cm CT and WDS showed higher amount of seeds, and WDS at 10-20 cm. As the sampling depth increased, NT showed fewer amount of seeds, while less reduction of the soil seedbank was observed in WDS. CT and WDS spread viable seeds in the soil profile from 0 to 20 cm depth. After 22 years there are viable weedy rice seeds up to 10 cm of depth in the three cropping systems and there is no difference among them up to 5 cm of depth, demonstrating the serious problem of the seedbank for this species.
Key words: no-tillage, conventional, wet direct-seeded, deterioration, depth.

Sobrevivência e distribuição do banco de sementes de arroz daninho após 22 anos de cultivo de arroz em diferentes sistemas

RESUMO: O arroz daninho (*Oryza sativa* L.) é a principal planta daninha do arroz cultivado e, devido à similaridade genética com a cultura, são poucas as alternativas de controle. Uma possibilidade são os sistemas de cultivo, em razão da influência na emergência e sobrevivência da espécie no banco de sementes do solo. Diante disso, o trabalho objetivou avaliar a longevidade e a distribuição das sementes de arroz daninho no perfil do solo, após 22 anos de cultivo, sob diferentes sistemas. Os tratamentos foram arranjados em esquema fatorial, em que o fator A foi composto por três sistemas de cultivo, realizados consecutivamente durante 22 anos, sendo esses: semeadura direta (SD), sistema convencional (SC) e pré-germinado (PG); e, o fator B composto por quatro profundidades de amostragem: 0-2, 2-5, 5-10 e 10-20 cm. As variáveis avaliadas foram: o número de sementes íntegras e deterioradas m^{-2}; e, a porcentagem de viabilidade. Não foi verificado efeito dos sistemas até 5 cm, mas a 5-10 cm SC e PG mostraram maior quantidade de sementes, e PG a 10-20 cm. A SD proporcionou diminuição de sementes íntegras conforme aumentou a profundidade de amostragem, enquanto o sistema PG elimina menos sementes do banco de sementes do solo. Os sistemas SC e PG distribuem sementes viáveis no perfil do solo de 0 a 20 cm de profundidade. Após 22 anos, nos três sistemas de cultivo há sementes de arroz daninho viáveis até 10 cm de profundidade, sendo igual a quantidade de sementes viáveis até cinco centímetros de profundidade.
Palavras-chave: semeadura direta, convencional, pré-germinado, deterioração, profundidade.

INTRODUCTION

Weedy rice (*Oryza sativa* L.) is the main weed in flooded rice fields (SOSBAI, 2018), due to the inefficient control by herbicides because of the similar genetic characteristics with cultivated rice (CHAUHAN, 2013) and also the resistant gotten by gene flow from Clearfield rice production system (AVILA et al., 2021). Another important strategy to control weedy rice is the flooding. However, it was reported weedy rice biotype tolerant to flooding (KASPARY et al., 2020), increasing the weed competitive ability and reducing crop yield. Therefore, weedy rice has been reported as a highly competitive and harmful weed to the cultivated rice, with losses from 6% to 49%, depending on the biotype (SHIVRAIN et al., 2009).

In lowland seedbanks the weedy rice is becoming the main species (MASSONI et al., 2013). Some of the reasons are the early seed shattering that starts at 25% of moisture content, which allows a continuous replacement of the soil seedbank, making

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weed management more problematic (DOMÍNGUEZ et al., 2009). Also, primary and secondary dormancy in weedy rice are other survival mechanisms that increase the crop management issues (SHIVRAIN et al., 2009).

The dynamic, density and botanic composition of the soil seedbank is a balance between new entries and withdrawals (CHAUHAN & JOHNSON, 2009). In general, a higher reduction of the seedbank is expected at lower depths, as a consequence of higher seed exposition to appropriate conditions for germination or factors associated with senescence. However, most of the weed seeds are located at the soil surface or shallow depths; because there are more entries in the soil surface due to new inputs from seed shattering (BENVENUTI, 2007; MASSONI et al., 2013).

Weed soil seedbank can be modified promoting germination and senescence by the use of cropping systems that change the composition and density. Minimum tillage, conventional tillage (CT) and wet direct-seeded (WDS) are the most accepted cropping systems for lowland rice cultivation in Southern Brazil (ULGUIM et al., 2021). The system which rice is sown directly into the soil not tilled, since the harvest of the previous crop, it is called no-tillage (NT) and less used, except for studies of conservation systems (KASCHUK et al., 2010; FAROOQ et al., 2011).

Most of the previous research in soil management practices, crop rotation and crop systems for weed seedbank viability has been developed for short (1-3 years) or middle (8-10 years) terms (SHIVRAIN et al., 2009; MASSONI et al., 2013; SINGH et al., 2015); consequently little information is known about the behavior of weed seedbank and patterns of viability at the field. Thus, more research is needed to increase the knowledge about survival, viability and emergence of weeds from the soil seedbank in long term, which is crucial to establish new and adequate management strategies to control weeds, according to patterns over long terms. Therefore, this research assessed the viability and vertical seed distribution of weedy rice in the soil profile after 22 years of different cropping systems.

MATERIALS AND METHODS

Site description

This experiment was conducted for 22 years at Cachoerinha (latitude 29º56'51"S, longitude 51º07'13"W, and 17 masl) in Rio Grande do Sul, Brazil, using a randomized complete block design with three replications. The experiment started in the 1994/95 growing season. Each block measured 40 m x 28 m, and the total plot area was 1,120 m^2. The soil was classified as Entisol Fluvents, and the crop management was made according to the flooded rice manual for field practices (SOSBAI, 2018). Weed management, according to growing season, was made by pre and post-emergence herbicides. Weedy rice management consisted of pulling out weeds by hand; however, since the growing season 2003/2004 was controlled by Clearfield® cultivars in rotation with no-Clearfield® cultivars. In both cases there was no total weed control which allows weed seed production and entries into the soil seedbank.

Cropping systems and sampling depth

This study compared the seedbank of three kind of cropping systems (NT, CT and WDS) in rice for 22 years, in combination with four sampling depths (0-2 cm, 2-5 cm, 5-10 cm and 10-20 cm). The field experimental area was cultivated with rice under three different treatments: NT, CT and WDS systems. In the NT system, the soil was not harrowed, plowed, or otherwise mechanically treated to minimize the disturbance. For CT system, the soil was leveled and tilled before planting to provide conditions favorable for early rice plant development. Conversely, in WDS system, germinated seeds (physiological stage of germination) were sown in a previously flooded soil which was tilled intensively (even more than CT) with a rotary hoe (SOSBAI, 2018). In winter and springtime, ryegrass (*Lolium multiflorum*) was cultivated on the experimental plots, sown in autumn and burndown for rice sowing. The average ryegrass dry mass was ~3,500 kg ha^{-1} over time.

During the growing season 2016/17, six soil sub-samples per replication from each depth were collected with a soil column cylinder of 400 cm^3. A high-pressure washer was attached to a special seed extracting equipment to wash each sample and separate the seeds from the soil (FERRERO & VIDOTTO, 1997), with a set of sieves of 4.75 mm; 2.38 mm; 1.41 mm; 1.19 mm and 1 mm. Only the material retained in sieves 4.75 mm and 2.38 mm were considered for the study. Then, samples were dried for 12 days at 30ºC and weedy rice seeds were carefully separated and sorted manually.

Seed quality assessment

The number of whole and deteriorated seeds per m^{-2}, and viability percentage were assessed. Seeds that preserved the caryopsis intact were considered whole seed. Broken, senesced and decayed seeds (commonly only with palea and lemma) were defined as deteriorated seeds.

The viability of the seeds were determined by the number of whole seeds determined with the tetrazolium test (2, 3, 5 triphenyl chloride salt of tetrazolium). Seeds were soaked in water for 18 hours; subsequently, a longitudinal cut through the seed coat bisecting the embryo was made and half was retained for staining with 1% of tetrazolium solution at 30ºC, in a glass container and covered with aluminum foil for two hours (BRASIL, 2009). Viability percentage was calculated based on the number of whole and viable seeds.

Data analysis

Data was analyzed as a two-way factorial, with cropping systems as one factor and sampling depth (0-2 cm, 2-5 cm, 5-10 cm and 10-20 cm) as the other factor. Response variables were tested for normality and homoscedasticity of the variances with the Shapiro-Wilk and Hartley tests, respectively. Normality test did not show a normal distribution of the means, so data was transformed with the equation log10 (x + 1) to reach normality, where "x" was the observed value for each unit of observation. Then, data was analyzed with ANOVA procedure to detect difference between the two factors and means were compared by the Tukey's test ($P \leq 0.05$). Additionally, correlation analysis was performed to compare the number of whole seeds with deteriorated seeds and percentage of viability in response of the cropping systems.

RESULTS AND DISCUSSION

Statistical analysis showed interaction between the factors for the three variables: whole seeds, deteriorated seeds and viability (Figure 1 A, B and C). No effect between the systems were detected up to 5 cm of depth, however at 5-10 cm for CT and WDS showed higher amount of seeds, and WDS at 10-20 cm of depth exhibited the highest value (Figure 1A). SINGH et al. (2015) reported that NT in rice has higher proportion of weed seeds from 0 to 2 cm of depth, and as depth increases, CT is the one that exhibits more seeds. These results were consistent with our research, and corroborate the outcomes at 10 cm of depth, which could be due to the continued use of CT and WDS systems for 22 consecutive years, which allowed a soil profile disturbance and seed burials at greater depths.

Different weed species of rice and the tillage with CT and WDS systems allowed the vertical distribution of weed seeds in the soil profile from 0 to 20 cm of depth (CHAUHAN & JOHNSON, 2009). However, specifically for CT, part of the weed seedbank can be easily exposed on the soil surface, which promotes germination and emergence, with the subsequent elimination by tillage or non-selective herbicides (CHAUHAN, 2013). Also a higher loss of longevity is expected in CT due to a faster deterioration process by the exposure of the seeds to unfavorable conservation conditions such as desiccation-hydration and predation (SINGH et al., 2015). In our research these observations were reported for CT and WDS systems, that showed more whole seeds (Figure 1A) but also more deteriorated seeds at lower depths (Figure 1B), as it is exhibited in the figure 2A, by the correlation analysis between this two variables.

The seed deterioration for the WDS system may be related to the fact that in WDS part of the tillage and sowing is made in flooding conditions, and very soon after sowing it is common to drain or not to replace the water level in order to stimulate crop establishment. However, this soil management system allows the germination of some weeds like weedy rice (CHAUHAN et al., 2015), which explains why most of the weedy rice seeds germinated. Thus, this practice reduces the weedy rice seedbanks, but eventually the same area could be infested again by the emerged plants, if they are not controlled properly.

Weed problems have increased seriously in Asia due to re-infestation of weedy rice, because of the frequent use of WDS system (CHAUHAN et al., 2012). In this sense, important efforts have been done to encourage the use of soil conservation systems in the main rice production areas where NT is the key for success. NT system is based on sowing without any disturbance of the soil structure, unlike the CT and WDS systems, which basically prepare the soil before sowing, altering the composition and dynamics of the weed seedbank (SINGH et al., 2015). Another cause of seedbank increasing is the weedy rice resistance to herbicides due to difficult control and rising of seed rain (AVILA et al., 2021). However, this was not an element that could affect the results of our research, because there was no evidence of weed resistance to herbicide at the field experimental area infested, thus the differences observed were an exclusively effect from the tillage system tested. Also, it is important to understand that the continues use of the same system tillage, such as NT, is not recommended for long time because of some problems in crop establishment during very wet or very dry spells and reduction of reliability due to poorly structure and compaction of the soil (SOANE et al., 2012).

Soil disturbance and tillage in CT buries the weed seeds at greater depths, while NT allows more seeds on the soil surface (DOMÍNGUEZ et al.,

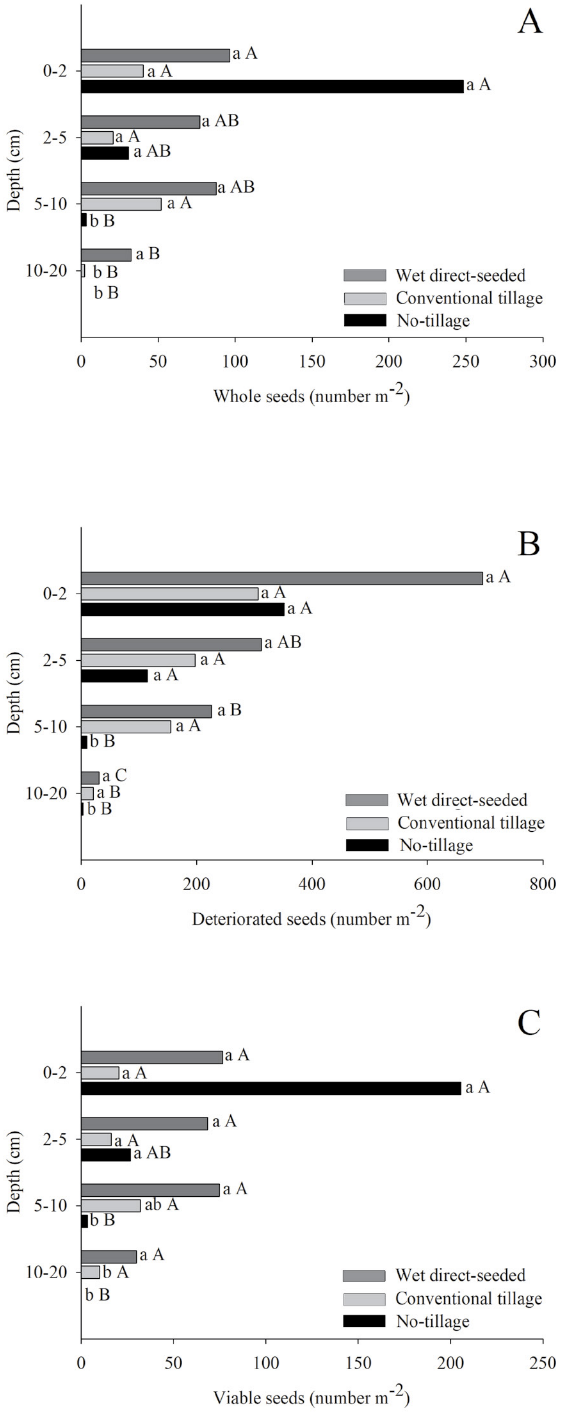
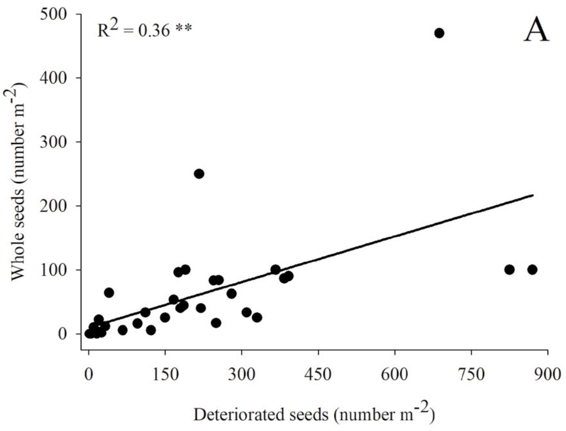


Figure 1 - Number of whole (A), deteriorated seeds per m^{-2} (B) and viability (C) of weedy rice, in response to four sampling depths and cropping systems carried out for 22 years [no-tillage (NT ■), conventional tillage (CT ■), wet direct-seeded (WDS ■)]. Bars followed by the same lowercase letter show no difference within the same depth comparing cropping systems; and the same capital letter indicates no difference within the cropping system comparing depths, by the Tukey test (P ≤ 0.05).



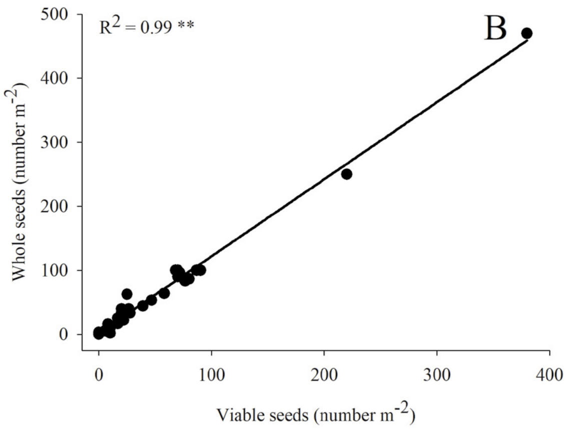


Figure 2 - Correlation between the number of whole seeds with the variables deteriorated seeds (A) and viable seeds (B), in response to four sampling depths and cropping systems carried out for 22 years [no-tillage (NT), conventional tillage (CT), wet direct-seeded (WDS)]. Each point is a replication for each cropping system (n = 36). Asterisks * and ** represent significance at 5 and 1% probability level, respectively. ns = there was no statistical difference.

2009). As observed in this experiment, a decrease of whole seeds by NT was detected as the sampling depth increased, and no seeds were found at 10-20 cm of depth (Figure 1-A). The highest number of deteriorated seeds in the NT system was reported from 0 cm to 5 cm, which can be related to germination and predation because of higher biological activity at this depth range.

This research demonstrated that the WDS system buried the seeds at greater depths, besides is a management practice that destroys less weedy rice seeds from the seedbank, and shows higher seed viability at 10-20 cm (Figure 1-C). The WDS system is characterized by the land leveling process, since final tillage and sowing are performed under flooding (TAO et al., 2016), condition that does not promote breaking seed dormancy and germination in weed seeds, keeping the seeds viable in the seedbank. Also no seeds were reported in NT at 10-20cm, so this explains the seed viability results for this system at this specific depth.

When soil preparation is finally done in the WDS system, the land is flooded for 20 days before sowing, and the crop is kept under flooding for most of the cycle. In this condition, weedy rice seeds that remain between the water-soil interphase can germinate and emerge from the soil, however seeds buried at 1 cm of depth from the soil surface, and under the same flooding level, do not germinate because changes in temperature in these conditions are not enough to be detected by the seeds to start the germination process (CHAUHAN et al., 2012), as observed in this study.

The results comparing soil depths showed a reduction for all systems in the number of whole seeds at depths greater than 10 cm, except for NT which exhibited this behavior from 5 cm (Figure 1-A). However, in NT and WDS more deteriorated seeds were found from 0 cm to 5 cm, while CT showed more deteriorated seeds from 0 cm to 10 cm (Figure 1-B). Also, as soil depth increased, the number of whole and deteriorated seeds decreased, as confirmed by the positive correlation between both variables (Figure 2-A), indicating the opposite relationship between these process, regardless of the system. This agrees with DOMÍNGUEZ et al. (2009) which found more rice seeds at 0-10 cm of depth compared to the range of 10-20 cm.

In general, no differences were found in the evaluated parameters of this study at the range of 2-10 cm of depth, which may be associated with the capacity of weedy rice to emerge from 7 cm (CHAUHAN et al., 2012), and to the primary and secondary dormancy that weedy rice seeds can develop; characteristics that avoid germination, and increase seed longevity and persistence of the seedbank for 7 years (SHIVRAIN et al., 2009). As well, positive correlations were found for whole seeds with deteriorated and viable seeds (Figure 2) thus, as the number of whole seeds increased, the number of deteriorated and viable seeds increased. Similar results were reported by DOMINGUEZ et al. (2009), which showed data of viable and deteriorated seeds equivalent with data exhibited in our study.

The CT and WDS systems showed higher amount of seeds at the high depth of 10-20 cm, which increases the longevity of weedy rice seeds in the soil (NOLDIN et al., 2006). Thus, in case of soil disturbance, this characteristic of the weedy rice suggested a subsequent problem of weed management for the next cropping seasons. Moreover, regardless of the rice cropping system there are viable weedy rice seeds up to 10 cm of depth after 22 years, demonstrating the serious problem of the seedbank for this species. Therefore, weedy rice seed viability is not completely lost by the implementation of NT, CT and WDS systems for 22 years, especially when the seedbank is resupplied by seed rain. Rotation between crop rice systems could be the strategy to reduce and keep low densities of weedy rice seedbank. Therefore, sowing the first year using NT, then (second year) with CT or WDS, to prevent the weedy rice seedbank from increasing on the soil surface, and to avoid conditions that promote seed longevity, as the burial at greater depths.

CONCLUSION

The implementation of the NT system reduces the number of whole seeds as the burial depth increases and the WDS system eradicates less seeds from the seedbank than the other two systems. Also, the CT and WDS systems spread viable weedy rice seeds in the soil profiles from 0 cm to 20 cm of depth, which increases seed deterioration in the superficial soil layers due to tillage when is compared with buried seeds. After 22 years of management there are viable weedy rice seeds in any cropping system up to 10 cm of depth and up to 5 cm the amount of seeds is equal for all the systems.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS’ CONTRIBUTIONS

Conceptualization: Agostinetto, D.; Carlos, F.S.; Ulguim, A.R. Data acquisition: Vargas, A.A.M.; Cereza, T.V. Data analysis: Vargas, A.A.M.; Ulguim, A.R. Design of methodology: Carlos, F.S.; Ulguim, A.R.. Writing and editing: Vargas, A.A.M.; Agostinetto, D.; Ulguim, A.R.

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