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Quantitative and qualitative analyses of weed seed banks of different agroecosystems

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One of the main survival mechanisms of weeds in constantly disturbed environments, specially the annual weeds, is their high production of seeds. In this study it was intended to evaluate the influence of different agroecosystems (vegetable garden, pasture, native field, soybean, dry bean and corn) on the strength of the seed bank, making quantitative and qualitative analyses. On each site, soil samples were collected, split to submit half to seed extraction by washing samples with water and counting the total number of seeds (quantitative analysis), and half to germination in trays placed in a greenhouse to evaluate weed emergence (qualitative analysis). The quantitative analysis of the agroecosystems showed that those cultivated with corn and vegetable garden presented best conditions for weed occurrence. The qualitative analysis resulted in the highest number of viable seeds for the vegetable garden (141,094,713 seeds, of which 74,965,862 were from monocotyledons plants and 66,128,851 dicotyledons). The weed seed concentration found for the vegetable garden is probably related to the management intensity in the area. The inverse is observed for the environments of less management intensity, as pasture and native field. Dry bean and soybean plots presented small seed bank and low emergence.

Key words: Vegetable garden, pasture, soybean, dry bean, corn, native field.

INTRODUCTION

The reserves of viable seeds in soil at the surface and in depth are known as seed bank (Gomes and Christoffoleti, 2008), other concepts or designations being also found. It is also known as seed reservoir, including the amount of non dormant seeds and other plant propagation structures present in the soil or in plant residues (Monquero and Christoffoleti, 2003). This reserve is the sum of all produced and introduced seeds along time that continue alive and dormant, with the seeds recently produced (Kuva et al., 2008). The variability and botanical density of a seed bank at a given time are the result of the balance between the input of new seeds

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Table 1. Treatments and sampling collection sites.

Treatments	Soil sampling location
1	Vegetable Garden
2	Soybean Field at harvest[1]
3	Dry bean Field at harvest[1]
4	Corn Field at harvest[1]
5	Native Field
6	Pasture

[1]area of Grass-legume rotation under no tillage.

(by rain and dispersion) and losses by germination, deterioration, parasitism, predation and transport out of the area (Machado et al., 2013).

An accurate measure of weed emergency is a subsidy to farmers for a more efficient weed control without the inappropriate use of herbicides (Kuva et al., 2008). The quantitative and qualitative evaluation of seed banks can practically be made only by direct germination in soil samples and by physical and chemical seed extraction followed by viability essays (Luschei et al., 1998). The size and the composition of the weed seed bank are very important for the decision of integrated weed management strategies. The *in situ* observation of seedling emergence in the field may give a general indication of the size and composition of the vegetative population, and of the seed bank. However, this is not a precise method because several seeds can stay viable for a long period without germination, and some of the germinated seeds may not emerge due to unfavorable conditions or to deep positioning in the soil (Lacerda et al., 2005). The simplification of the environment that characterizes the modern agricultural systems, as for example mono-cropping, accelerates the ecological succession patterns (Gasparino et al., 2006), generating specialized “habitats” within ecosystems.

The cultivation system exerts an influence on the size of the seed bank. Carmona (1995) estimated the seed banks of four distinct agroecosystems: crop rotation (soybean, fallow, dry bean); lowland; citrus orchard and pasture of *Brachiaria brizantha*. The average quantities of seed per square meter were 22,313 for lowland; 6,768 for rotation; 3,595 for the orchard crowns; and 529 for pasture. He also found out that similarities of seed bank sizes among agroecosystems is greater for the most disturbed areas, as it is the case of crop rotation, lowland and orchards. In agricultural areas seed banks are comparatively greater than in non agricultural areas of low environmental disturbance, because weeds have a strategy of producing large numbers of seeds in much disturbed situations (Monquero and Christoffoletti, 2005). Environmental and management factors influence the seed consumption rate by predator organisms (Balbinot et al., 2002). The consumption of these seeds is made by a large number of species (animals, insects, fungi, etc) that are naturally present in the environment.

In agroecosystems the weed population is related to their seed bank, so that the knowledge of the seed bank size and of its species composition can be used to predict future infestations, to construct population models along time, and consequently for the definition of management programs that lead to a better rationalization of the use of herbicides (Gardarin et al., 2011; Soltani et al., 2013). In general, the decision-making of weed management strategies is based on visual evaluations of the needed weed control intensity without much technical criteria. It is therefore important, for emerging technologies like precision agriculture, to develop control strategies based on estimations of the potential of the weeds in the soil. They should be supported by research and be based on economical viability (Voll et al., 2003).

One of the important factors in studies of seed banks is related to the techniques used for their evaluation (Caetano et al., 2001). The quantification of a cultivated soil seed bank includes the problem of the minimum number of soil samples to be collected in order to have a precise estimation of the number of seeds per unit area (Voll et al., 2003). The understanding of the dynamics of a weed seed bank and the simulation of the emergency flux are among the most recent strategies used for weed control (Vivian et al., 2008). In this context, this experiment was carried out with the aim of evaluating the influence of different types of soil use, that is, different agroecosystems on their seed banks, making qualitative and quantitative analyses.

MATERIALS AND METHODS

Experiments were carried out in Ponta Grossa, PR, Brazil, where the climate is sub-tropical humid, mesotherimic, of Cfb type (Koeppen, 1931), with mild summers and frequent frosts in winter. Average temperature of the hottest month is less than 22°C and of the coldest month is less than 14°C. Average yearly rainfall is 1,545 mm, with no defined dry season (IAPAR, 2008). The soil is a red latosol, typical distrophic, according to EMBRAPA (2006), and an Oxisol according to the Soil Survey (2010). Natural vegetation is dominated by C_4 plants represented by some grasses like *Andropogon* sp., *Aristida* sp., *Paspalnm* sp., *Panicum* sp., and gallery forests are found along the natural drainage canals. The relief is softly ondulated with slopes between 2 and 7%. Data were collected during two different phases, first collecting soil samples for the qualitative analysis, and second manipulating tray soil samples for the quantitative analysis of the seed banks. The sites for sample collection, which represent the treatments, belong to different environments as shown in Table 1.

The fields of dry bean, soybean and corn where soil samples were collected, were cultivated by a long duration minimum tillage system with a rotation schedule shown in Table 2. The experimental field design was completely randomized with six treatments and 16 replicates (plots of 3 × 6 m), carried out as described above. Soil samples were composed of 20 sub-samples of each agroecosystem from the surface layer (0 to 0.5 m) in a randomized way. One composite soil sample of 3 to 4 kg was prepared after homogenizing the sub-samples, which were divided into parts A and B.

Sample A: This was used for the quantitative analysis of the seed bank through the number of seeds per ha and per 0.05 m of soil

Table 2. Rotation schemes where dry bean, soybean and corn soil samples were collected.

Season-year	Rotation scheme			
Winter - 2000	AP + E[1]	AP	T	P
Summer - 2000/2001	M[2]	S/F	F/S	S
Winter - 2001	AP[3]	T	P	AP+E
Summer -2001/2002	S/F[4]	F/S	S	M
Winter - 2002	T[5]	P	AP+E	AP
Summer - 2002/2003	F/S[6]	S	M	S/F
Winter - 2003	P[7]	AP+E	AP	T
Summer - 2003/2004	S[8]	M	S/F	F/S
Winter - 2004	AP/E	AP	T	P
Summer - 2004/2005	M	S/F	F/S	S

[1]Black oat + pea, [2]Corn, [3]Black oat, [4]Soybean/dry bean, [5]Wheat, [6]Dry bean/soybean, [7]Fallow, [8]Soybean.

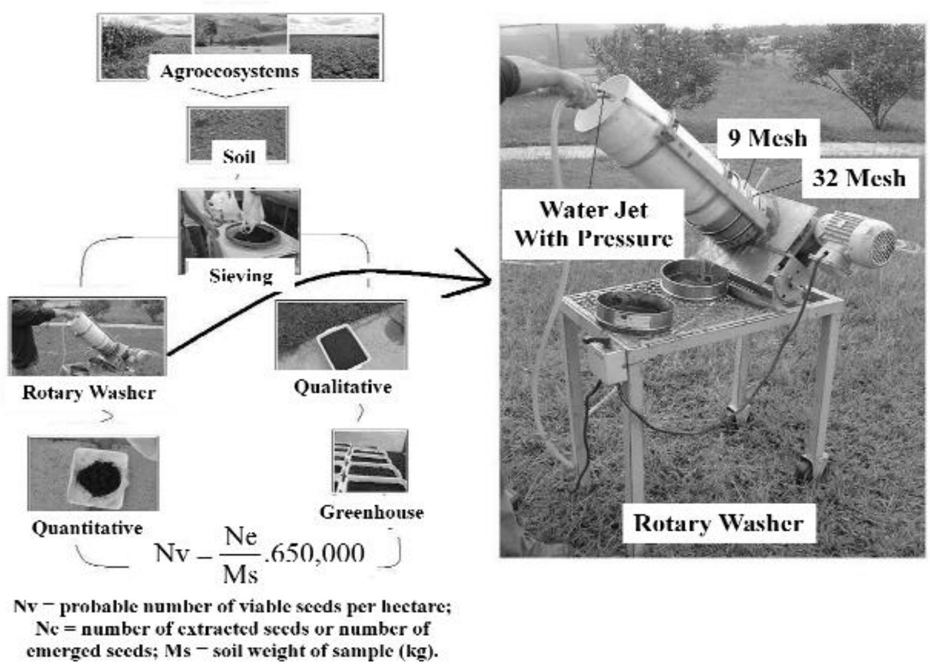

Figure 1. Visual description of the used methodology.

depth. Samples were passed through a rotary washer (Figure 1) with two sieves, one with larger openings (9 mesh) to retain coarser materials like plant residues, and one with smaller openings (32 mesh) to retain seeds, coarser soil particles and aggregates, and plant material that was not retained by the previous sieve. After washing samples were air dried for 20 days and, with aid of a magnifying glass the inert material was separated and seeds counted.

Sample B: This was used for the qualitative analysis evaluating the number of non dormant viable seeds (germinated and emerging later) per ha and 0.05 per m depth. For the qualitative analysis field soil samples were displayed on plastic trays to form a 0.04 – 0.05 cm soil layer, so that seed depth would not be a limiting factor for their germination. Trays were displayed in a greenhouse and irrigated periodically to allow the germination of non dormant seeds. At fixed time intervals emerged seedling were counted, making the distinction of the monocotyledons and dicotyledons. These seedlings were eliminated to give place to those germinating later. When the germination flux ended, soil was turned over to stimulate further germination, and counts continued for 53 days, a time admitted sufficient for this evaluation. The quantitative analysis was based on the estimative of the total number of probable seeds per hectare, per 0.05 m layer in depth, and the qualitative analysis on the estimative of the number of probable viable seeds in the same layer. For this layer of average soil bulk density of 1.3 g cm^{-3}, the soil mass totalized 650,000 kg of dry soil. The probable number of seeds per hectare in the 0.05 m soil layer was calculated through Equation (1) of Monqueiro and Christoffoleti (2003):

$$Nv = \frac{Ne}{Ms}.650,000 \tag{1}$$

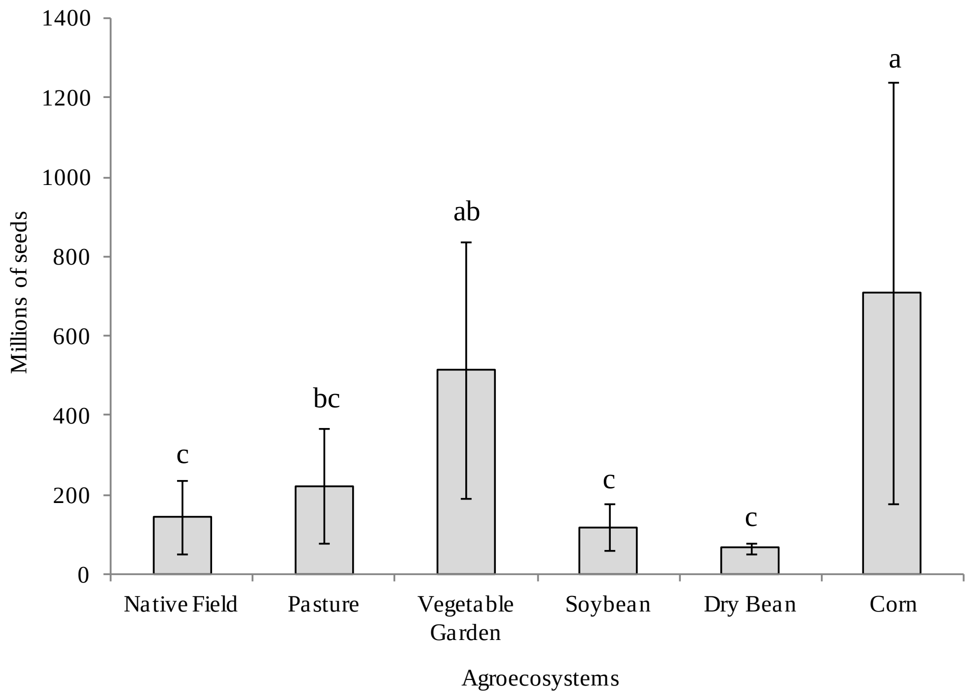

Figure 2. Seed bank size per hectare – quantitative analysis. Averages followed by the same letter do no differ among each other by the Duncan test at 5% significance level.

Where: Nv = probable number of viable seeds per hectare; Ne = number of extracted seeds or number of emerged seeds; Ms = soil weight of sample (kg).

Results were submitted to analysis of variance and means were compared by the Duncan test ($p< 0.05$), using the SASM - Agric (2001) program.

RESULTS AND DISCUSSION

In a general way, data show a great variability in both, the seed banks as well as the total germination of these banks in the different ecosystems. According to Carmona (1995), such variability is normal in these types of studies. The largest weed seed bank was found in the agroecosystem of corn (Figure 2), however not statistically different from the vegetable garden. The agroecosystem pasture presented the third largest seed bank, also not differing from the other, followed by native field, soybean and dry bean.

The history of the experimental fields (Table 2) points to a winter fallow interval that may have favored a renovation of the seed bank since weeds could complete their reproduction cycles. However, there was no difference between the agroecosystems corn and vegetable garden. This happened due to a better relation between the environment (soil and climate) and the weed species present in this environment that had a better ability to contribute to the establishment of a seed bank. In relation to the vegetable garden agroecosystem, soil revolvement stimulates an increase of seed viability. Practices that promote the inversion of soil layers as plowing, foment a better seed distribution within the soil profile, and also bury a significant amount of seeds so that the regeneration capacity of part of certain seed populations is derailed. On the other hand, practices that do not invert soil layers allow the majority of the seeds to remain at soil surface (Lacerda et al., 2005). According to Lacerda et al. (2005), higher values of weed viable seeds in the conventional management system is due to the frequent soil perturbations by mechanical implements during a corn field establishment in summer.

For the pasture agroecosystem, the lack of soil perturbation added to the low fertility, promoted a more stable environment that is propitious only for few species with less individuals, reducing the strength of the seed bank (Carmona, 1995). Marquezan et al. (2003) analyzing the dynamic of a red rice seed bank, concluded that during the fallow period rice seed was reduced on average by 85% per year because the soil surface seeds lost their viability more rapidly in relation to deeper seeds. Another explanation is in the way data obtained in small samples are transformed to hectares through the average relation of soil mass per unit volume. Ideally, soil bulk density should be measured along sampling points in order to have more representative data. Observing again the history of the area (Table 2), now in relation to the agroecosystems soybean and dry bean, we can see a much lower seed number in comparison to the vegetable

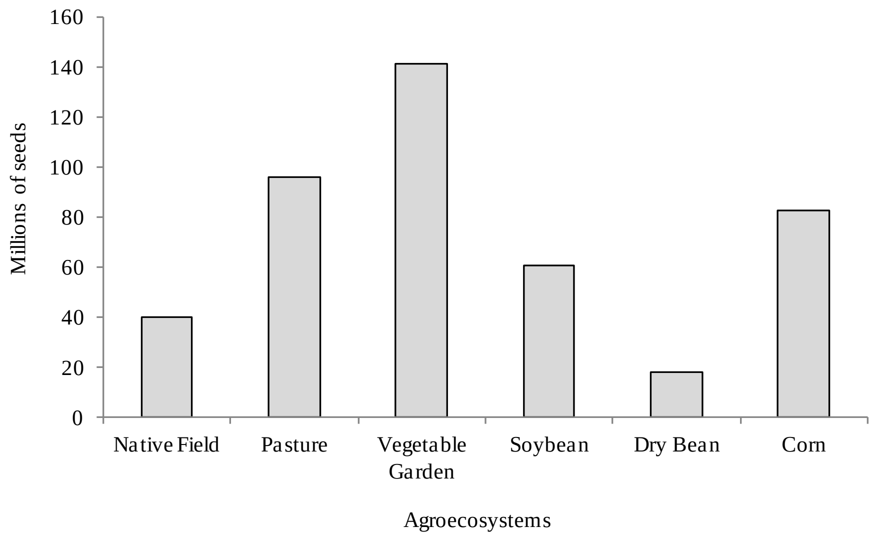

Figure 3. Probable total number of viable seeds (mono and dicotiledons) per hectare – qualitative analysis**.**

Table 3. Percentage of dormant seeds and emerged seeds, in relation to the total number of the seed bank.

Agroecosystem	Dormant seeds (%)	Emerged seeds (%)
Native field	72.19	27.81
Pasture	57.08	42.92
Vegetable garden	72.63	27.37
Soybean	48.54	51.46
Dry bean	73.40	26.60
Corn	88.30	11.70

garden and corn (Figure 3). This is probably due to the rapid and good covering of the soil surface by the crop and use of herbicides, which do not favor weed germination. This is the case of pastures and native fields because according to Caetano et al. (2001) the application of herbicides for weed control influences the distribution of the seed bank in the soil profile.

The variability of the data obtained in this type of study is normally high due to the relatively great non uniformity of seed distribution in the soil (Carmona, 1995). Observing the number of viable seeds (Figure 3) obtained from seeds germinated in soil trays, we can see little differences between treatments. However, observing the vegetable garden environment in relation to the other, it can be noted that the viable seed bank of this environment is much larger. This is mainly due to the fact that this environment involves manual control of weeds, little use of herbicides, constant soil revolvement, in this way favoring the renovation of the seed bank. This is confirmed when looking at the dry bean environment that due to soil surface shading and use of herbicides, diminishes drastically the renovation of the soil seed bank.

Despite the obtained data, it can be seen in Table 3 that most of the seeds remain dormant for variable periods. To analyze a dormant seed bank there is need of longer evaluations. However, observing the total seed number together with the number of viable seeds, it can be noted that the viability potential of the seeds is only manifested when stimulated and submitted to ideal conditions of development. Dormancy and its seasonal changes are related to the persistence of seeds in the soil and, consequently, to the problems faced during the infestation of the crops. Weed seeds pass through annual cycles of more or less intensity of dormancy. These changes are attributed to variations in temperature, light, rainfall, agricultural practices and seed depth (Vivian et al., 2008). For the vegetable garden environment (Table 3), 72.63% of the seeds are dormant. This is due to soil mixing that lead to a more uniform seed distribution in the profile, and in a burying of a greater amount of seeds making them unviable (Lacerda et al., 2005). For the

Table 4. Qualitative analysis of mono and dicotyledon weeds in the surface layer (0.05 m of deep).

Agroecosystems	Probable number of viable seeds	
	Monocotyledons	dicotyledons
Native field	22360435[ab]*	17903565[b]*
Pasture	75402000[a]	20353111[b]
Vegetable Garden	74965862[a]	66128851[a]
Soybean	40002667[ab]	20958000[b]
Dry bean	11017804[b]	6859021[b]
Corn	58793967[ab]	24020134[b]

*Averages followed by the same letter do no differ among each other by the Duncan test at 5% significance level.

other agroecosystems the percent of dormant seeds was also high since seeds can remain viable in the soil for long periods without germination (Caetano et al., 2001). In this respect, Lacerda et al. (2005) states that in fallow areas the number of species and viable seeds in the soil are smaller. For all agroecosystems the majority of the weeds were monocotyledons (Table 4). For the corn environment the development of monocotyledons was favored because corn itself is a monocotyledon and herbicides used in this situation did not control weeds from the family Poaceae. For the agroecosystem vegetable garden, a large number of monocotyledons were observed, which can be explained by the intense cultivation of this area, with several stimulations of the seed bank. Blanco and Blanco (1991) observed that the weed management through soil movement with rotary hoes stimulated weed seed emergence.

For the dry bean environment the development of monocotyledons was also favored, despite being a dicotyledon plant. In this environment, however, there was an equilibrium of viable mono and dicotyledons seeds because besides soil chemical and physical effects, there were biological effects due to the interference of plant residues on the survival of seeds from the bank (Gomes and Christoffoleti, 2008) taking into account that the crop was managed under minimum tillage. For the natural ecosystem of native fields, an even greater equilibrium between viable mono- and dicotyledon seeds would be expected. This is however explained by the fact that the monocotyledon seed population was larger than that of the dicotyledons.

Cultivation systems favored the renovation of seed banks, with different intensities, and promoted a better development of monocotyledons in relation to dicotyledons. The size and composition of the soil seed banks are extremely variable among different habitats (Kuva et al., 2008). This is a response of the strategy of invasive plants producing a large number of seeds and having good dissemination mechanisms, longevity and dormancy to survive in hostile environments. The evaluation of the need for control of weeds is a function of the emergence rate of the species present in the soil seed bank and has to be established for each management system of the implanted crop (Voll et al., 2003).

In general, seed banks are composed of many species, few of them dominant, corresponding to 70 to 90% of the total seed number in the soil. These species are considered harmful because they resist control measures and are more adaptable to different climatic conditions.

Conclusions

The emergence of monocotyledon plant seeds was greater in all agroecosystems, especially in corn and pasture. The agroecosystem vegetable garden favored the increase of the soil seed bank because of its more intense soil revolvement and less use of chemical weed control. The agroecosystems dry bean and soybean presented low emergency and a smaller seed bank.

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