Restoration Ecology
THE JOURNAL OF THE SOCIETY FOR ECOLOGICAL RESTORATION INTERNATIONAL

RESEARCH ARTICLE

Establishment of Fabaceae Tree Species in a Tropical Pasture: Influence of Seed Size and Weeding Methods

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Abstract

The successful establishment of trees in pastures may be related to species, seed size, and weed control treatments. This study experimentally evaluated the emergence, establishment after 2 years, and growth of individuals of 7 tree species with different seed sizes sowed under three weeding methods (no weeding, grasses clipped, and grasses removed). The experiment was carried out in a tropical abandoned pasture in Midwestern Brazil, dominated by the exotic invasive grass *Urochloa brizantha*. The effect of seed size on seedling emergence was significant and was more intense in the no weeding and weeding treatment plots than in grasses clipped. Furthermore, an increase in seed size resulted in an increase in the probability of establishment of tree species, although this pattern also differed among weeding treatments. The increase in seed size reflected negatively on tree growth in the absence of grasses, whereas in the presence of grasses there was an inverse relationship. The experimental results suggested that the use of seeds of different sizes for direct seeding in pastures is a possible strategy of Fabaceae tree species reintroduction in pastures within the Cerrado. Fabaceae species with seeds larger than 100 mg can establish in areas with no weeding, whereas species with seeds smaller than 50 mg can establish preferentially in areas with weeding, growing faster than larger ones. Thus, weeding strategies can determine the success of direct seeding of different species in restoration projects of tropical pastures.

Key words: **direct seeding, exotic grass competition, seedling emergency, seedling survival.**

Introduction

Tropical ecosystems have been largely converted to pastures and degraded, and abandoned pastures are a common situation. In the Brazilian Cerrados (tropical savanna vegetation type), a consequence of pasture area conversion is the invasion of native areas by exotic grasses, such as *Melinis minutiflora* Beauv., *Brachiaria decumbens* Stapf., and *Panicum maximum* Jacq., which can limit the growth of native tree species populations (Pivello et al. 1999). Thus, the restoration of these degraded environments is necessary to restore regional biodiversity. Fabaceae tree species attract great interest in restoration projects, mainly because these species fix nitrogen from the atmosphere (Siddique et al. 2008), contribute to soil recovery, and increase biological activity, thus increasing the probability of establishment of other tree species less tolerant to the stressful conditions of degraded areas (Franco et al. 1992).

Some studies pointed out that the presence of herbaceous vegetation in seed deposition sites can facilitate the establishment of tree species by maintaining a favorable microclimate for seed germination and seedling establishment (Posada et al. 2000; Vieira & Scariot 2006). However, several experimental studies removing grasses from plots have shown that the surrounding vegetation, especially native and exotic grasses in pastures, can inhibit the growth and survival of seedlings by competition (Rey Benayas et al. 2003; Craven et al. 2009; García-Orth & Martinéz-Ramos 2011). However, whether competition with the grass is more intense between shoots or roots remains little studied. In one of the few studies reported in the literature for pasture areas, Holl (1998) showed that competition for resources between *Calophyllum brasiliense* and an invasive grass was more intense above than belowground. Thus, one of the main post-dispersal barriers that influence seedling establishment in pastures seems to be competition with grasses (Hooper et al. 2002; García-Orth & Martinéz-Ramos 2011) and the establishment of trees in pasture areas varies widely among species and may be related to seed size (Camargo et al. 2002; Hooper et al. 2002; Peterson & Carson 2008; Sovu et al. 2010).

Some components of the life history of plants, such as seed size and germination phenology, influence species recruitment patterns (Peterson & Carson 2008; Sovu et al. 2010). Species with larger seeds have greater chances of establishment than those with smaller seeds in stressful conditions imposed by

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doi: 10.1111/j.1526-100X.2011.00858.x

factors, such as competition, drought, shading, and herbivores (Leishman et al. 2001; Moles & Westoby 2004). However, they are not observed frequently in stressful areas such as pastures. Doust et al. (2006) questioned if the rarity of individuals of species with large seeds (which are characteristic of late-successional stages) in these environments would only be the result of dispersal limitation.

This study experimentally assessed the effects of different weeding methods of exotic grasses (no weeding, clipping of grasses and removal of grasses) in the establishment and performance of seven tree species with different seed sizes regarding (1) emergence; (2) establishment (survival after 2 years) and (3) growth of shoots. Furthermore, we asked if the grass competition effect is more intense above or belowground. We expected that regardless of the weeding method, larger seeds would have a higher probability of establishment because of their higher performance under stress, and the establishment of species from smaller seeds would be influenced by weeding methods.

Methods

Study Area

This study was carried out in an experimental pasture area composed by the grass *Urochloa brizantha* (Hochst. Ex A. Rich) R. D. Webster belonging to Embrapa Gado de Corte, in Campo Grande district, Mato Grosso do Sul State, Midwestern Brazil (20°25′27″S 54°41′16″W). The average annual precipitation for the region ranges from 1,400 to 1,600 mm, and the regime of precipitation is typically tropical, with two distinct periods: a rainy season which starts in October and extends through March and a dry season during the period April to September. The months with higher rainfall are January with an average rainfall 150–250 mm. The driest month is July with an average of 10 to 40 mm. The average annual temperature varies between 22 and 25°C. The hottest month is October, with temperatures between 23 and 27°C, and July is the coldest, with temperatures ranging from 17 to 22°C (Campelo-Júnior et al. 1997). The relief is characterized by plains, with altitude between 200 and 600 m. The soil is clay dystrophic red latosol. The area was originally occupied by Cerrado sensu stricto, a vegetation type typically dominated by shrubs, small trees, and an herbaceous layer dominated by grasses. Fifteen years before the experiment was set up, the area was deforested and the vegetation was completely replaced by the exotic grass *U. brizantha.*

Experimental Design

To evaluate the effects of species with different seed sizes and weeding method on the establishment of tree species, we carried out a randomized blocks design experiment in areas of abandoned pasture. The factor "Seed Size," expressed by seed mass (g), was used as a covariate, whereas the factor "Weeding" was comprised by three levels of treatment. In the first level, "Grasses removed with chemical weeding" (GR), the grasses were totally removed by application of the herbicide glyphosate; in the second level, "Grasses clipped" (GC), the vegetation was clipped at about 10 cm above the ground; in the third level, "Control" (CT), the grasses were left to grow freely. The seed size gradient was composed by seven species of Fabaceae that occurs in forests within the region of the study area (Salomão et al. 2003) (Table 1). We used seven species of Fabaceae with different seed sizes but similar in other traits (Table 1).

Five blocks (sites) were randomly marked in the pasture area, at least 20 m distant from each other to incorporate some of the pasture environmental heterogeneity. Within each site, three plots (10 × 7 m each) were marked, separated by 5 m from each other to avoid the influence of neighboring plots. Each plot was randomly allocated to a grass management level. Within each plot, 70 subplots (1 m^2 each) were marked, and each species was seeded in 10 randomly determined subplots. To increase the chances that at least one individual would emerge to be monitored, five seeds were planted in each subplot, about 10 cm from each other. The seeds were buried to reduce the effects of predation (Doust et al. 2006), at a depth of approximately three times its thickness (smaller dimension). Thus, each site contained a combination of all treatments, seed size and management of grasses. A total of 5,250 seeds from the seven species (750 seeds from each species) were sown in

Table 1. Ecological traits of the species used in the experiment, and treatment for overcoming seed dormancy.

Species	*Ecological Category*	*Seed Size (mg)**	*Treatment to Overcoming Seed Dormancy*
Mimosa caesalpiniifolia Benth.	Pioneer	24	Chemical scarification in H_2SO_4 (7 minutes) (Martins et al. 1992)
Peltophorum dubium (Spreng.) Taub.	Mid-successional	46	Soaking in hot water (80°C during 1 minute) (Oliveira et al. 2008)
Pterogyne nitens Tul.	Mid-successional	180	Coat punction (Nassif & Perez 1997)
Dimorphandra mollis Benth.	Pioneer	220	Mechanical scarification (Salomão et al. 2003)
Copaifera langsdorffii Desf.	Late-successional	470	Chemical scarification in H_2SO_4 (5 minutes) (Salomão et al. 2003)
Dipteryx alata Vog.	Mid-successional	1,200	No dormancy
Hymenaea stigonocarpa Mart.	Late-successional	3,900	Mechanical scarification (Pereira et al. 2011)

All species are neutral photoblastic, heliophyte, and drought tolerant.
* Seed size means were estimated according to Brasil (2009), by dividing the mass of one thousand seeds by 1,000.

the five sites, 15 plots, and 1,050 subplots. When more than a seedling emerged and survived for over 4 months, the exceeding seedlings were carefully removed to avoid intraspecific competition effects.

To avoid confounding the effects of competition with grasses and seasonality on survival rates, we assured that seedlings of different species emerged during the same period by subjecting dormant seeds to pre-germinative treatments before the experiment (Table 1). With the exception of *Dipteryx alata*, all species have dormant seeds, with impermeable seed coats, and received appropriated treatments to overcome seed dormancy (Table 1).

The seeds were sown on 26 January 2009, and all plots were sampled every 15 days until the end of the rainy season (4 months), monthly until 12 months from the beginning of the experiment, and bimonthly in the subsequent months, until the experiment completed 2 years. All individuals were tagged to certify that no individuals from other seed sources were included in the analyses. At the end of the experiment, we recorded the number of emerged seedlings and established saplings, and stem base diameter (SBD) of the plants. The plants that survived until the end of the 2-year period were regarded as established.

The experimental set up was maintained by cutting the grass shoots in the GC treatment when the grasses overcame 20 cm height, and by carefully removing by hand new grasses resprout in the GR treatment. The interval between maintenance services was determined by field observations, and was more frequent in the rainy season (monthly) than in the dry season (every 2 or 3 months).

To determine the viability of the seed batch of each species used in the field experiment, we carried out a control experiment in a nursery. We used four replicates of 50 seeds for each species, which were previously subject to the same pre-germinative treatment described above. The seeds were sown in styrofoam trays containing Plantmax® (DDL Agroindustria, Paulínia, SP) substrate and maintained in the nursery, and were moistened 10 times a day during 10 minutes using a spray watering system with an outflow of 7 L/hour. We counted the number of emerged seedlings every 15 days for 3 months.

Data Analysis

We used logistic regression analyses to evaluate seedling emergence and survival in the field experiment, and seedling emergence in the nursery experiment following Jaccard (2001). To evaluate the effects of seed size on seedling emergence in the nursery experiment, the model included the following terms: seed size and squared seed size as independent variables, and the binary response (emerged versus not emerged) as the dependent variable. The squared term of seed size was included because of its significance, in the greenhouse and in the field (see "Results" section). The logistic regression model used to evaluate the effects of seed size and weeding methods of grasses on sapling establishment did not include the site effect term as it was not significant ($\Delta\chi^2 = 14.371$, $df = 4$, $p > 0.75$).

The influences of seed size (continuous variable) and weeding method (categorical variable) on plant SBD after 2 years growing in the experiment were evaluated with analysis of covariance (ANCOVA). As the block (site) effect was not significant ($p > 0.50$, for site main effect and their interactions), it was not included in the model. All regression models were significant (see "Results" section), so we used seed size as a covariate to evaluate the effect of weeding method. The interaction between seed size and weeding method was significant, so we used the HSD-Tukey multiple comparisons test to compare the slopes of the regression lines. Plants that emerged from small seeds of *Mimosa caesalpiniifolia* and *Peltophorum dubium* did not survive after 2 years in the GR treatment, and were excluded from the analysis for this treatment.

The significance level used in the analyses was $p < 0.05$. Residuals were graphically checked to evaluate trends along the estimates. Data for seed size and stem basal diameter were transformed to natural logarithms to obtain homogeneous variances (Sokal & Rolph 1995). All analyses were carried out using the Systat 10.2 software (Systat 2002).

Results

The probability of seedling emergence in the nursery experiment reflected seed viability and varied with seed size, with a positive squared term. The smallest and largest seeds had higher probabilities of seedling emergence, whereas intermediate-sized seeds, such as *P. dubium*, *Pterogyne nitens*, and *D. mollis* had lower probabilities (Table 2, Fig. 1a). In the field experiment, the general pattern for each treatment level reflected the seed viability patterns shown in the nursery experiment, with a significant, positive effect of the squared seed size term. However, the curves adjusted by the logistic regression model differed among the three levels of grass management (Table 2, Fig. 1b–d). In the grass clipped treatment (GC), there was a lower variation in the probability of seedling emergence relative to seed size, as indicated by the lower value of the squared term coefficient (Fig. 1c), whereas for the other two treatment levels (CT and GR) there is a clear advantage of the larger seeds on seedling emergence, followed by the smaller seeds (Fig. 1b & 1d). The curves of the treatment levels CT and GR did not differ significantly from each other (Table 2), whereas the curves were significantly different between GC and CT, and between GC and GR (Table 2, Fig. 1a).

The probability of establishment (survival after 2 years of sowing) was also influenced by seed size and levels of weeding (Table 3, Fig. 2a). However, this pattern differed among the weeding treatment levels. There was no significant difference between the curves of the CT and GC treatment levels (Table 3, Fig. 2b & 2c), whereas significant differences between GC and GR, and between CT and GR were found (Table 3, Fig. 2b–d). There was a larger influence of seed size on plant establishment in the GC and CT levels, when compared to the GR level (Fig. 2a).

Within each treatment level, the SBD was strongly related with seed size (Fig. 3a–c). The SBD of the plants after

Table 2. Results of the logistic regression analyses evaluating the effects of seed size on seedling emergence for each weeding method level, and contrasts between distinct weeding methods.

				β Coefficient	
Treatments	*General model* χ^2	*df*	*Constant*	*Seed mass*	*Seed mass*^{2}
Nursery experiment	78.06***	2	5.914	−2.079 ± 0.215***	0.212 ± 0.033***
Field experiment					
Control	48.71***	2	1.842	−1.029 ± 0.215***	0.102 ± 0.019***
Grasses clipped	9.78**	2	0.838	−0.498 ± 0.210**	0.048 ± 0.018**
Grasses removed	108.87***	2	1.795	−1.125 ± 0.219***	0.121 ± 0.019***
Contrasts					
Grasses clipped versus control			1.005 NS	0.532 ± 0.301 NS	−0.054 ± 0.026*
Grasses removed versus control			−0.047 NS	−0.095 ± 0.307 NS	0.019 ± 0.027 NS
Grasses removed versus grasses clipped			0.957 NS	−0.627 ± 0.304*	0.073 ± 0.027**

NS, not significant.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

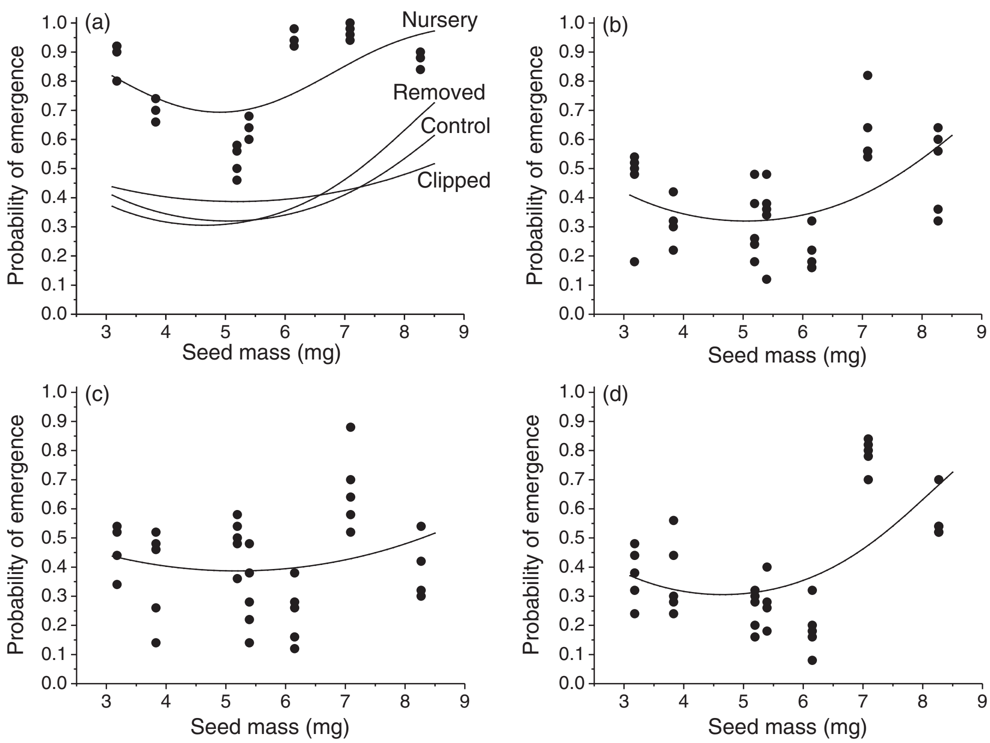

Figure 1. Seedling emergence responses to seed size: expected curves in the nursery experiment and in three weeding method levels of the field experiment (a), in control plots (b), in grasses clipped plots (c), and grasses removed plots (d). Data for seed size transformed to natural logarithms.

2 years of sowing was influenced both by seed size and grass management treatments (ANCOVA, interaction between seed size versus treatment; $F_{2,460} = 117.88$, $p < 0.001$, Fig. 3a). In the treatment levels CT (Fig. 3b) and GC (Fig. 3c), the increase in seed size resulted in a significant increase in plant SBD. When grasses were removed, however, plants with higher seed size presented smaller SBD (Fig. 3d).

Discussion

The emergence of tree species that disperse to degraded areas is primarily limited by the availability of safe sites for seed germination which, specifically in pasture areas, can be determined by competition with exotic grasses (Doust et al. 2006; Flory & Clay 2010). This study showed that the probability of emergence of tree species in pastures varied among species and was related to seed mass and weeding methods.

We expected to find a positive linear relationship between the probability of emergence and increases in seed size (Peterson & Carson 2008). However, our results showed a quadratic relationship between seedling emergence and seed size. The pattern found in the field experiment reflected the general pattern described for seed viability obtained from the nursery experiment, so that intermediate-sized seeds used in

Table 3. Results of the logistic regression analyses evaluating the effects of seed size on plant survival after two years of seeding for each weeding method level, and contrasts between distinct weeding methods.

Treatments	*General Model* χ^2	*df*	*β Coefficient* *Constant*	*β Coefficient* *Seed Mass*
Control	178.53***	1	−9.463	1.486 ± 0.169***
Grasses clipped	157.38***	1	−7.266	1.205 ± 0.133***
Grasses removed	19.68***	1	−0.791	0.392 ± 0.093***
Contrasts				
Control versus grasses clipped			2.197 NS	0.282 ± 0.216 NS
Control versus grasses removed			—	1.095 ± 0.193***
Grasses clipped versus grasses removed			—	0.813 ± 0.163***

NS, not significant.
*** $p < 0.001$.

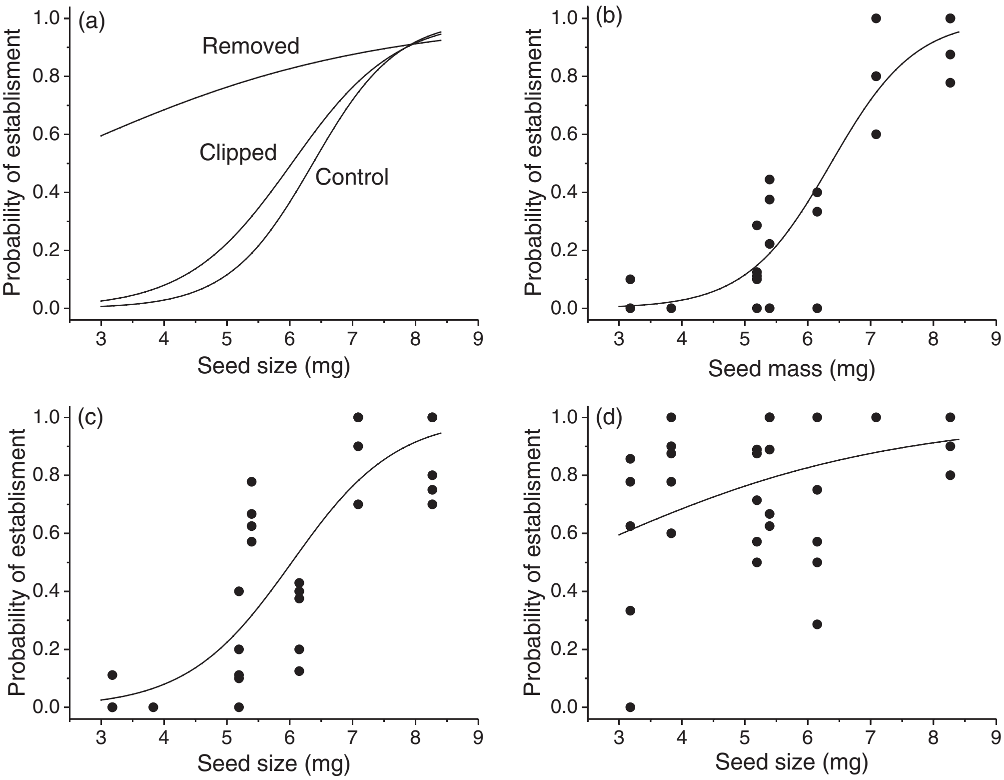

Figure 2. Plant establishment responses to seed size: expected curves in the three weeding method levels of the field experiment (a), in control plots (b), in grasses clipped plots (c), and grasses removed plots (d). Data for seed size transformed to natural logarithms.

this experiment had a lower probability of emergency than smaller and larger ones.

The shape of the seedling emergence curves varied between the different levels of weeding methods, as shown by the significant interaction between weeding method and seed size. When grasses were clipped, the coefficient of the quadratic term was lower than for the other two treatments (CT and GR), suggesting a clear advantage of the largest seeds on seedling emergence in control and removed grass treatment levels. Exogenous factors that primarily affect the germination of seeds are light, temperature, oxygen, and water (Bradford & Nonogaki 2007). The species used in the experiment were all neutral photoblastic, they germinate in a wide range of temperatures in the tropics (between 15 and 35°C, Brancalion et al. 2007) and they were not subjected to low levels of oxygen by excess of water; however, moisture availability in the topsoil may have been the main factor affecting seedling emergence. The treatments CT and GR may have exposed seeds to a more stressful environment through the reduction of soil superficial layer moisture, available to seeds, due to increased exposure of soils in the GR treatment, and to a greater water uptake by grasses in CT. Thus, the increased soil water deficit stress to which these seeds were submitted resulted in an increase in the advantage of larger seeds, whereas in less

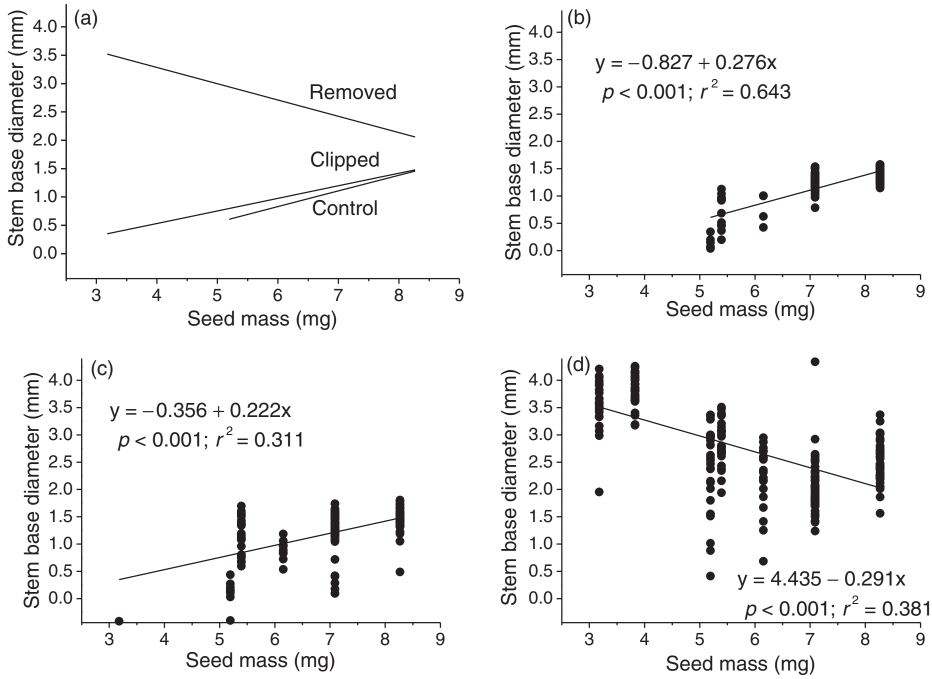

Figure 3. Stem base diameter responses to seed size: expected curves in the three weeding method levels of the field experiment (a), in control plots (b), in grasses clipped plots (c), and grasses removed plots (d). Data for seed size and stem basal diameter transformed to natural logarithms.

stressful environments, where evapotranspiration was reduced by clipping the shoots (GC treatment), this relationship became weaker.

Plant survival after 2 years was strongly influenced by seed mass and the weeding method, but the adjusted logit model relating plant survival and these two variables was linear and not quadratic as found for seedling emergence. In the presence of grasses (GC and CT treatments), there was a strong increase in the probability of sapling establishment with increasing seed mass. In the absence of grasses, this effect was less pronounced, although the increase in seed size also resulted in increased probabilities of plant survival.

Rey Benayas et al. (2003) evaluated the effects of competition with herbaceous plants on the establishment of *Quercus faginea*, through a controlled experiment that measured soil moisture between 15 and 45 cm depth in the absence of herbs, with herbs clipped to 8 cm above the ground and with intact herbs growing freely. Their results showed that water availability was affected by the herb management treatments, so that water availability was higher in the absence of herbs than herbs clipped and herbs intact. The reduction in water availability was highly related to seedling mortality of *Quercus faginea*, suggesting that the negative effects of herbaceous vegetation were primarily a result of competition for water. Our results also suggest that competition between the tree species and the grasses may have occurred mainly for belowground resources, as clipping grass shoots did not result in significant increases in sapling establishment when compared with control plots, whereas the removal of grass roots in the GR treatment significantly increased the probability of establishment, specially of species with smaller seeds.

This study, however, did not determine which of the belowground resources, nutrients and/or water, restricted the establishment of the tree species studied. However, as the Cerrado has a well-defined dry season, it is possible that the establishment of tree species in pastures in this region is constrained by the water deficit stress in the dry season, which is magnified by competition with grasses. Henkin et al. (1998) suggested that the success of seedling establishment of shrubs in Mediterranean climate (where a well-defined dry season is also found) reflected mainly the accessibility of water below the root zone of herbaceous vegetation.

The size of seeds has been considered of special importance in the early stage of seedlings, as there is strong evidence that high reserve supply in larger seed species would be favorable to their establishment (Camargo et al. 2002; Metz et al. 2010). However, the relationship between seed size and survival is poorly evaluated in other stages of the life cycle of plants, especially in long-lived species.

The advantage of large seeds would be higher only during the initial phases of the establishment—a period when the cotyledons remain attached to the plants—and tend to disappear when the seed reserves have been completely exhausted (Leishman et al. 2001; Moles & Westoby 2004). Our results, however, showed that the effect of initial reserves available for seedling establishment increased their chances of survival for at least 2 years, period much longer than the abscission of the cotyledons. It is possible that this has occurred due to a greater root development that enabled the tree species to penetrate below the main rooting zone of the grasses and reach the water deeper in the soil, increasing therefore the probability of establishment. Some studies found a positive relationship between sapling survival and the investment in roots to reach greater depths of soil (Lloret et al. 1999; Padilla & Pugnaire 2007); furthermore, the development of a larger root system may be related to seed size (Lloret et al. 1999). Leishman and Westoby (1994) suggested that seedlings from larger seeds may have a larger root absolute size than seedlings originating from smaller ones, which would allow an advantage to access water in deeper soil layers, and that larger seeds supply their seedlings with a greater metabolic reserve than small seeds, enabling tolerance to drought and low nutrient levels. Thus, the probability of establishment under conditions of water restriction, as occurs in competition with grasses, could be related to the amount of seed reserves and consequent large roots, resulting in a higher probability of establishment of species with larger seeds as shown in this study.

The influence of seed size in SBD varied among the different levels of weeding methods, because in the absence of grasses an increase in seed size resulted in smaller plants, whereas in the presence of grasses the inverse relationship occurred. The results relating the growth of plants and different weeding methods of grasses once more suggest that competition among tree species and grasses occurred primarily for belowground resources and not for light. If light had limited tree species growth, we would expect to find a bigger difference in plant growth between treatments CT and GC. However, this did not occur and a smaller difference in growth was observed between the GC and CT treatments than between GR and the other treatments. As all species used in this study are heliophytic, it is possible that the exotic grass shoots in the experiment were not able to completely shade the tree saplings and the resource light had a low or negligible influence on growth of tree species.

In humid tropical systems, the removal of roots and shoots of competing species can lead to increased growth rates of seedlings (Holl 1998; Hooper et al. 2002). The relative growth of seedlings tends to be negatively correlated with seed size (Leishman et al. 2001). Our results found this relationship in the absence of grass, where plants with small seeds, characteristic of fast growing species, would not have resource constraints and grew faster than plants with large seeds, characteristic of slow-growing species. However, under competition, we found that species with larger seeds grew more than species with smaller seeds, possibly a result from differences in the available seeds reserves. In fact, Hooper et al. (2002) found a pattern similar to our results, showing that many tree species with smaller and medium seeds grew at lower rates in relation to those with larger seeds under competition with grasses than in the absence of competition. Baraloto et al. (2005) proposed that species with smaller seeds tend to have higher growth potential and may overcome the seedlings from larger seeds, especially when there are no resource constraints.

Restoration projects of degraded forest areas are usually made by planting tree seedlings (Doust et al. 2008; García-Orth & Martinéz-Ramos 2011). Direct seeding, however, may represent an attractive alternative, being more easily applied and with much lower costs (Engel & Parrotta 2001; Camargo et al. 2002). However, most studies using direct seeding were conducted in temperate regions (Doust et al. 2008), and the study and use of this method in tropical regions have been limited (Engel & Parrotta 2001; Peterson and Carson 2008). Although the present study tested the seeding of only seven species, our results suggest the possibility of restoring Cerrado areas by direct seeding, at least with seeds in the same range of masses used here. Thus, other studies that expand the number of species and study areas can contribute to the use of direct seeding in these regions.

Implications for Practice

- The use of direct seeding can be a possible and efficient strategy to forest recovery projects in abandoned pastures in Cerrado regions, especially by Fabaceae species with seed mass larger than 100 mg. As the cost of direct seeding can be smaller than introduction of seedlings, it may be an attractive strategy at larger scales.
- In pastures of Cerrado areas, weeding procedures that include weed root removal can increase the probability of establishment of a greater number of species with different seed sizes.
- Within the Fabaceae species studied, the species with smaller seeds may offer some advantages over those with larger seeds in terms of early growth rates.

Acknowledgments

We thank Fundação de Apoio ao Desenvolvimento Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT) for financial support, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship to S.R.P.

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